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Proteins, Polypeptides, Amino Acids

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In this issue:

- **NUCLEOPROTEINS** 1-2
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** 3-9
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** 10-24
- **BINDING/ RECEPTOR PROTEINS** 25-26
- **MEMBRANE/ SURFACE PROTEINS** 27-34
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** 35-37
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** 38-44
- **DIETARY AND NUTRITIONAL EFFECTS** 45
- **POLYAMINES - SYNTHESIS AND METABOLISM** 46
- **OTHER RELATED STUDIES** 47-53

NUCLEOPROTEINS

1. BINDING OF BENZO(A)PYRENE DERIVATIVES TO SPECIFIC PROTEINS IN NUCLEI OF INTACT HAMSTER EMBRYO CELLS.

MacLeod MC, Kootstra A, Mansfield BK, Slaga TJ, Selkirk JK
Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Cancer Res; 41(10):4080-4086 1981

In hamster embryo cells incubated for 24 hr with 4 uM [3H] benzo(a)pyrene, a major portion of the nonextractable radioactivity in nuclear preparations copurifies with the protein fraction. When these proteins are analyzed by sodium dodecyl

HE 20.3173/2

sulfate:polyacrylamide gel electrophores is, significant variations in the labeling intensities of the various proteins are seen. Control experiments demonstrate that the labeling is due to covalent binding to protein. Histones H3 and H2A are heavily labeled while the other histones of the nucleosome core, H2B and H4, are devoid of radioactivity. Large amounts of label are associated with proteins with mobilities similar to the very lysine-rich histone H1. However, the results of differential extraction experiments suggest that the labeled proteins do not belong to either the H1 or the high-mobility-group class of chromosomal proteins. During 6 hr of inhibition of protein synthesis by cycloheximide, the metabolism of [3H]benzo(a)pyrene, as monitored by high-pressure liquid chromatography, remained normal. Patterns of labeling of nuclear proteins after 3 or 6 hr were identical in the presence and absence of cycloheximide. This finding strongly suggests that binding of benzo(a)pyrene derivatives to nuclear proteins occurs in situ. (Author abstract) (55 Refs)

2. TISSUE SPECIFICITY AND CELLULAR DISTRIBUTION OF NOVIKOFF HEPATOMA ANTIGENIC PROTEINS P39, P49, AND P56.

Schmidt WN, Stryjecka-Zimmer M, Glass WF, Briggs RC, Hnilica LS
Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
J Biol Chem; 256(15):8117-8123 1981

Three Novikoff hepatoma protein antigens (approx Mr = 39,000, 49,000 and 56,000) were partially purified from Novikoff hepatoma chromatin. Rabbit antiserum to these three proteins was used to examine various rat tissues for the presence of these antigens. Immunological specificity of the antiserum was assessed using quantitative microcomplement fixation assay or by visualization of the immunoreactive complexes with peroxidase-antiperoxidase procedure after transferring the electrophoretically separated proteins to nitrocellulose sheets. The immunoreactivity was localized with the three proteins p39, p49, and p56 in Novikoff hepatoma. The p56 protein was found to be present in normal rat liver, 24-hr regenerating rat liver, fetal rat liver, or kidney, albeit in much smaller amounts as found in Novikoff hepatoma. The p49 and p39 antigens were specific for Novikoff hepatoma. Immunoabsorption experiments confirmed the specificity of this antiserum. Assessment of various subcellular fractions of Novikoff hepatoma revealed that the p39, p49, and p56 protein antigens are present in the cytoplasmic fractions as well as in isolated chromatin. (Author abstract) (34 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

3. IDENTIFICATION AND IMMUNOPRECIPITATION OF CYTOSKELETAL PROTEINS FROM MURINE EXTRA-EMBRYONIC ENDODERMAL CELLS.

Oshima RG
Dept. Biology, Massachsetts Inst. Technology, Cambridge, MA, 02139
J Biol Chem; 256(15):8124-8133 1981

Murine extra-embryonic endodermal cells derived from either teratocarcinomas or cultured mouse blastocysts contain two protein species of Mr = 55,000 and Mr = 50,000 endodermal cytoskeletal proteins A and B, respectively, that are insoluble in nonionic detergent and 1 M NaCl and are not found in abundance in embryonal carcinoma cells, the stem cells of teratocarcinomas. Antiserum raised against the electrophoretically purified endo B protein immunoprecipitated endo B from [35S]methionine-labeled cell lysates of three parietal endodermal cell lines, a presumptive visceral endodermal cell line, and a mouse hepatoma line. Immunoprecipitable endo B was not found in murine embryonal carcinoma cells, fibroblasts, myoblasts, keratinocytes, erythroleukemic or neuroblastoma cells. These results are consistent with the view that endo B is not tubulin, vimentin, desmin, or keratin. Amino acid composition data, partial peptide analysis of immunoprecipitated endo B, and immunoprecipitation analysis with antikeratin serum support the suggestion that endo B is not a keratin. Indirect immunofluorescent staining of parietal endodermal cells with the endo B antiserum resulted in the fluorescence of a fibrillar cytoskeletal network. The synthesis of endo B was increased dramatically when embryonal carcinoma cells were induced to differentiate by treatment with retinoic acid. Endo B appears to be a cytoskeletal protein that is synthesized when malignant embryonal carcinoma cells differentiate to benign extra-embryonic endoderm. (Author abstract) (71 Refs)

4. EPIDERMAL GROWTH FACTOR INDUCES REDISTRIBUTION OF ACTIN AND ALPHA-ACTININ IN HUMAN EPIDERMAL CARCINOMA CELLS.

Schlessinger J, Geiger B
Dept. Chemical Immunology, Weizmann Inst. Science, Rehovot, Israel
Exp Cell Res; 134(2):273-279 1981

We have examined the possibility that epidermal growth factor (EGF)-induced morphological changes in human epidermoid carcinoma (A-431) cells are related to a reorganization of specific cytoskeletal elements affected by the hormone. It was found that EGF induced striking changes in the distribution of actin and alpha-actinin within these cells. After 30-45 min of exposure to EGF there was a marked decrease in the degree of organization of the microfilament bundles and appearance of diffuse and punctuate labeling of actin and alpha-actinin. These effects were transient and upon prolonged incubation of 8 hr or more in the presence of EGF, the normal, well organized patterns of actin and alpha-actinin were restored. (Author abstract) (25 Refs)

5. HISTIOCYTOSIS X AND NERVE TISSUE-SPECIFIC S-100 PROTEIN (MEETING ABSTRACT).

Nakajima T, Motoi S, Sato Y, Watanabe S
Div. Pathology, Natl. Cancer Center, Chuo-ku, Tokyo 104, Japan
Nippon Monaikei Gakkai Kaishi; 21(1):87 1981

Paraffin-embedded preparations of tissues obtained from 2, 2 and 12 patients, respectively, with Letterer-Siwe disease, Hand-Schuller-Christian disease and eosinophilic granuloma were examined by the peroxidase-antiperoxidase enzyme-antibody method using anti-bovine S-100 protein rabbit serum to determine the distribution of S-100 protein in histiocytosis X. Purified S-100 protein-absorbed serum and normal rabbit serum were used as controls. S-100 protein was found in the giant histiocytic cells that proliferate in Letterer-Siwe disease, Hand-Schuller-Christian disease and eosinophilic granuloma. Most of the cells were stained strongly but some weakly, and unstained cells were also present. Such cells as lymphocytes and acidophils had no S-100 protein. Langerhans cells, interdigitating reticulum cells, and histiocytosis X were found to have immunohistologically common characteristics and to differ from normal histiocytes. Protein S-100 was considered valuable as a marker of nonphagocytic macrophages and histiocytosis X. (no Refs) (In JPN)

6. S-100 PROTEIN AS A NEW MARKER OF HISTIOCYTES (MEETING ABSTRACT).

Sato Y, Nakajima T, Watanabe S
Div. Pathology, Natl. Cancer Center, Chuo-ku, Tokvo 104, Japan
Nippon Monaikei Gakkai Kaishi; 21(1):104 1981

To determine the characteristics of the cells assumed to be histiocytes showing an S-100 protein-positive reaction, the presence or absence of S-100 protein in the histiocytes and monocytes in the bone marrow and blood of various tissues of the reticuloendothelial system was determined and compared immunocytochemically with other markers. In the superficial lymph nodes, cells containing S-100 protein were dispersed in the lymphatic sinus and paracortex area (T-zone region); these cells were stellate, having irregular projections. In the deep lymph nodes, there were generally few cells with S-100 protein. Tangible body macrophages at the active center had no protein S-100. The histiocytes containing S-100 protein generally had no lysozymes. In the spleen, only a few cells having S-100 protein were found in the white pulp. The granulocytes and monocytes in the bone marrow contained lysozymes but no S-100 protein. In the peripheral blood smear, lysozymes were found in granulocytes and monocytes, but there was no S-100 protein. From these findings, the S-100 protein-positive cells are assumed to have the same distribution as Langerhans cells and interdigitating reticulum cells. (no Refs) (In JPN)

7. DIFFERENCES OF EXPRESSION OF CYTOSKELETAL PROTEINS IN CULTURED RAT HEPATOCYTES AND HEPATOMA CELLS.

Franke WW, Mayer D, Schmid E, Denk H, Borenfreund E
Div. Membrane Biology and Biochemistry, Inst. Cell and Tumor Biology, D-6900 Heidelberg, W. Germany
Exp Cell Res; 134(2):345-365 1981

Cytoskeletal elements, enriched in intermediate-size filaments and insoluble in buffers of high salt concentrations and Triton X-100, were isolated from various cultures of rat hepatocytes and hepatoma cells, and their proteins were studied by one- and two-dimensional gel electrophoresis and immunofluorescence microscopy. The cells examined included several permanent cell lines (MH1C1, HTC, hepatoma 72/22, clone 12 from Gunn rat hepatocytes, and cell clones from normal rat hepatocytes), as well as freshly dissociated hepatocytes that were cultured and allowed to attach to substratum for increasing periods of time, beginning at 24 hr after removal of the liver from the animal. Filaments containing vimentin, which were not found in hepatocytes grown in liver tissue, were detected in most of the cultured hepatocytes and hepatoma cells, except in MH1C1 cells, and were shown to be newly synthesized during the first days of primary culture. Maintenance of expression of filaments containing proteins immunologically related epidermal prekeratin ('cytokeratins') was observed in all cells examined but HTC cells. Detailed comparison of the cytokeratin polypeptides present in various hepatocyte and hepatoma cell cultures showed that, in some of the cultured epithelial liver cells, cytokeratins are expressed which are identical with, or similar to, those of normal hepatocytes grown in the liver. On the other hand, differences in cytokeratin polypeptides were also found among different hepatocyte-derived cell cultures. Changes of expression of cytoskeletal proteins were found to occur even in cloned cell populations, and cells positive for certain cytokeratins could be seen next to other cells that were negative. The results demonstrate that profound changes of cytoskeletal composition, especially concerning intermediate filament protein patterns, can occur during culturing in vitro. Moreover, we show that intermediate filament protein patterns, can occur during culturing in vitro. Moreover, we show that different intermediate filament proteins can be expressed in different hepatocyte-derived cell cultures and that changes of cytoskeletal composition can occur in a given cell population, without obvious effects on cell growth rate and cell morphology. During culturing of hepatocytes and hepatoma cells, there seems to be a general tendency to induce the production of vimentin filaments as well as to maintain the production of cytokeratins similar to the hepatocyte-specific cytokeratins in liver tissue. However, the demonstrated exceptions speak against a role of these filament proteins as prerequisites for the growth of an epithelial cell in vitro. Rather, the presence of filaments containing certain cytokeratins and of desmosomes in epithelial cells growing in vitro seems to reflect the synthesis of specific differentiation markers which may be lost, independently, in some cells during culturing. (Author abstract) (56 Refs)

8. A COMPARATIVE STUDY OF THE IN VITRO POLYMERIZATION OF TUBULIN IN THE PRESENCE OF THE MICROTUBULE-ASSOCIATED PROTEINS MAP2 AND TAU.

Sandoval IV, Vandekerckhove JS
Div. Biology, California Inst. Technology, Pasadena, CA, 91125
J Biol Chem; 256(16):8795-8800 1981

At concentrations of microtubule-associated proteins MAP2 and tau inducing max microtubule formation, MAP2 promotes higher rates and higher levels of microtubule nucleation than tau. Microtubules polymerized to steady state by tau show a two-fold higher rate of tubulin loss compared to microtubules polymerized by MAP2. Microtubules polymerized in the presence of both MAP2 and tau contain lower levels of MAP2 (0.06 mol/mol of tubulin) and tau (0.07 mol/mol of tubulin) than microtubules polymerized in the exclusive presence of MAP2 (0.2 mol/mol of tubulin) or tau (0.25 mol/mol of tubulin). Addition of tau to microtubules polymerized by MAP2 results in incorporation of tau into microtubules (0.047 mol/mol of tubulin) and loss of MAP2 (0.1 mol/mol of tubulin). Similarly, incubation of microtubules polymerized by tau in the presence of MAP2 results in microtubule incorporation of MAP2 (0.1 mol/mol of tubulin) and loss of tau (0.08 mol/mol of tubulin). Microtubules and their ribbon precursor contain comparable levels of MAP2. Cross-sectional views of microtubules show no difference in the number of protofilaments (13 to 15) forming the MAP2 and tau microtubules. Fingerprints of cysteine-labeled tryptic peptides of two tau poly-peptides (tau4 and tau6) are very similar and differ totally from similar fingerprints of MAP2. (Author abstract) (28 Refs)

9. IDENTICAL 110,000 DALTON PHOSPHOPROTEINS IN NUCLEI AND POLYRIBOSOMES OF A RAPIDLY GROWING RAT HEPATOMA.

Kostka G, Schweiger A
Max-Planck-Institut fur Biochemie, 8033 Martinsried bei Munchen, W. Germany
Biochem Biophys Res Commun; 101(3):756-762 1981

Proteins derived from rat Morris hepatoma 3924 A cell nuclei and free polyribosomes were characterized. Whole hepatoma nuclei were incubated with 32P-ATP and proteins phosphorylated through the action of endogenous protein phosphokinases. HCl-soluble nuclear proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and examined by autoradiography. A large number of peptides in the mol wt range 10,000-150,000 Dalton, but relatively few phosphoprotein bands, were found. One of the major radioactive species had mol wt 110,000 Dalton. Free polyribosomes were labeled with 32P-ATP and dissociated with puromycin or EDTA, then fractionated on oligo[dT]cellulose columns and separated and analyzed like the nuclear proteins. Polyribosome proteins retained on the column and eluted with 25% formamide included two major species with mol wt 66,000 and 110,000 Dalton. Among the many ribosomal proteins phosphorylated during ATP incubation was the 110,000 Dalton protein. Coomassie blue stained peptides and phosphopeptides were obtained in a second SDS gel after limited proteolysis of the bands designated 110,000 Dalton. A complete correspondence between number and size of fragment bands derived from the nuclear and polyribosomal 110,000 Dalton component was found. Band mol wt ranged between 14,000 and 90,000 Dalton. The five major phosphopeptide spots identified by autoradiography were also identical for the 110,000 Dalton phosphoproteins, regardless of origin. The results show that the nuclei and free polyribosomes of a rapidly growing rat hepatoma contain an identical phosphoprotein with mol wt 110,000 Dalton. (16 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

10. FIBRONECTIN. IMMUNO-MORPHOLOGICAL STUDY OF ITS DISTRIBUTION IN HUMAN PATHOLOGY.

Birembaut P, Gaillard D, Adnet J-J, Dousset H, Kalis B, Poynard J-P, Leutenegger M, Labat-Robert J, Robert L
Laboratoire Pol Bouin, Service d'Histologie-Embryologie, Hopital Maison-Blanche, 45, rue Cognacq-Jay, F 51100 Reims, France
Ann Pathol; 1(2):140-146 1981

Fibronectin (FN) is a major glycoprotein component of extra-cellular matrix. This glycoprotein seems to be involved in cell to cell interaction and in cell to extracellular matrix interaction. Its distribution has been studied in various pathological conditions by an indirect immunofluorescent technique using a rabbit anti-FN serum. In various carcinomas, there is a loss of FN normally present within basement membranes, related to tumoral infiltration. Of particular interest is the irregular labeling of basement membranes in intraductal mammary carcinomas and comedocarcinomas and in noninfiltrating bronchial carcinoma. This discontinuous pattern seems to correspond to a potential neoplastic invasive process. In mammary benign lesions there is an inconstant and variable pericellular labeling around epithelial cells. This pericellular FN is never found in severe dysplastic and malignant lesions of the breast. The disappearance of this pericellular and basement membrane FN could be of practical interest in the detection of early malignant lesions. In benign connective proliferations FN is localized principally on cellular surfaces and vascular basement membranes. In sarcomas, there is a loss of the regular pericellular labeling around sarcomatous cells. These observations correlate well with cellular culture findings. Loss of pericellular FN seems to be related to malignant transformation. Detection of FN would be of diagnostic value in pseudo-tumoral or inflammatory lesions of the soft tissues. In the normal skin, FN is present in papillary dermis principally. In the skin of diabetic patients, there is an increase of FN in the superficial dermis and a frequent labeling of epidermis basement membrane. Thickened vascular basement membranes are also labeled by anti-FN serum. FN seems to be involved in the diabetic microangiopathy. In sclerodermic processes, there are qualitative and quantitative modifications of the distribution of cutaneous FN. FN is abundant in the lower dermis, especially in active fibrotic lesions. In the renal parenchyma, FN is found in the mesangium of glomeruli and in vascular basement membranes of the interstitium. FN is a good marker of the mesangium in numerous proliferative glomerulonephritis. FN is also linked to fibrin in thrombotic microangiopathy, epithelial crescents and fibrinoid necrosis. FN is not found in amyloid deposits. (21 Refs) (In FRE)

11. RAT IGD MYELOMA PROTEIN: CELL-FREE TRANSLATION OF THE DELTA MRNA AND BIOCHEMICAL ANALYSIS OF INTRACELLULAR AND MEMBRANE DELTA CHAIN.

Sire J, Alcaraz G, Bourgois A, Jordan B
Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, F-13288 Marseille Cedex 9, France
Eur J Immunol; 11(8):632-636 1981

The analysis of IgD molecules produced by the tumor-derived IR 731 cell line showed that these molecules are not secreted and are only present at the cell surface as covalent dimers of 48,000 dalton delta chains. The original IR 731 tumor secreted complete IgD; thus, the disappearance of secretion in the cell line was correlated with the absence of light chain synthesis. The mRNA coding for the cell line delta chain was isolated and translated. The analysis of the translated delta chain (mol wt: 40,000) confirmed the absence of a region having the size of a domain as previously shown by partial amino acid sequence. The observation that the absence in delta chain of this region, which comprises most of the CH2 domain, could be a general feature of murine species is discussed. (Author abstract) (48 Refs)

12. PARTIAL CHARACTERIZATION OF SIALOGLYCOPEPTIDES PRODUCED BY CULTURED HUMAN MELANOMA CELLS AND MELANOCYTES.

Bhavanandan VP, Katlick AW, Banks J, Kemper JG, Davidson EA
Dept. Biological Chemistry, Milton S. Hershey Medical Center, Pennsylvania State Univ., Hershey, PA, 17033
Biochemistry; 20(19):5586-5594 1981

The sialoglycopeptides produced by HM7 human melanoma and fetal uveal melanocyte cultures grown in the presence of [3H]glucosamine and [35H]sulfate were isolated from the Pronase digests of cells, spent media, and intracellular material. From the melanoma culture, six sialoglycopeptides, accounting for 43% of the total 3H radioactivity in the nondiffusible cell-associated glycopeptides, were purified. A major glycopeptide (GPIb) having an apparent mol wt in the range 12,000-15,000 showed specific sialic acid dependent interaction with wheat germ agglutinin (WGA). It was found to contain mainly O-glycosidically linked oligosaccharides having the structure (AcNeu)--o-2[Gal--GalNAc]; some N-glycosidically linked saccharides were also present. A second WGA-binding glycopeptide (GPIa) was smaller and less anionic and had a higher proportion of N-glycosidically linked saccharides than GPIb. The normal fetal cultures yielded either no (iris) or markedly reduced (melanocytes) quantities of the WGA-binding glycopeptides. The four WGA-nonbinding sialoglycopeptides purified from melanoma were shown to have complex (N-acetyllactosaminyl type) oligosaccharides linked via N-acetylglucosamine to asparagine with either no or insignificant amounts of O-glycosidically linked saccharides. The corresponding glycopeptides from melanocytes were of smaller molecular size and lower anionic charge, reflecting an overall lower degree of glycosylation. (Author abstract) (53 Refs)

13. IMMUNOLOGICAL STUDIES OF GASTRIC JUICE EXTRACTS FROM GASTRIC CANCER PATIENTS ON MITOGEN INDUCED LYMPHOCYTE TRANSFORMATION.

Ishigami H

First Dept. Surgery, Sch. Medicine, Chiba Univ., Inohana, Chiba 280, Japan

Nippon Geka Gakkai Zasshi; 82(3):230-236 1981

Under tetragastrin stimulation, gastric secretions were collected from 21 gastric cancer patients, 8 peptic ulcer patients and 8 healthy volunteers while keeping intragastric pH at 7. Consistent quantities of proteins were gathered from each of 10 gastric cancer patients, 8 peptic ulcer patients and 8 healthy volunteers, were mixed together by group and the mixtures were then named extract of gastric cancer secretions, extract of peptic ulcer secretions, and extract of healthy volunteer's secretions, respectively. The influence of 100 ug/ml protein of these extracts upon phytohemagglutinin (PHA), concanavalin A (Con A) or Sepharose protein A (SpA)-induced lymphoproliferative response of normal peripheral lymphocytes was evaluated by 3H-thymidine uptake into DNA. When the extract of gastric cancer secretions was first added to the lymphocyte culture with the mitogens listed above, the stimulation index (SI: ratio of cpm with mitogen and extract to cpm without mitogen) was significantly lower than the SIs obtained with the other two extracts. When the extract of gastric cancer secretions was added to culture at 2-hr intervals for 24 hr, the point of suppression of lymphoproliferation was observed to be in the early stage of lymphocyte transformation. Influence of the extract of gastric cancer secretions on mitogen-induced LP was also evaluated on the basis of the size of the tumors from which the secretions were obtained; suppressive activity was observed mainly in the extract from tumors 10-20 square cm. The results suggested that the extract of gastric cancer secretions, chiefly from tumor size 10-20 square cm, possesses suppressive activity on both T and B cells, and the point of suppression is in the early stage of lymphocyte transformation. (18 Refs) (In JPN)

14. THE EFFECT OF SEROMUCOID ON COAGULATION.

Klatzow DJ, Vos GH

Dept. Biochemistry, Univ. Durban-Westville, Durban, South Africa

S Afr Med J; 60(11):424-427 1981

The purpose of this study was to investigate the effect on coagulation of alpha-acid-glycoprotein, one of the acute-phase reactants, the blood level of which is elevated in malignant disease and various acute conditions. Alpha-acid-glycoprotein was obtained from serum and plasma of normal volunteers. The amount obtainable from serum was greater than that obtained from plasma (43 mg/dl v 22 mg/dl), and differences were noted in the mol wt of the two preparations; alpha-acid-glycoprotein from serum had a mol wt of 55,000, while that from plasma appeared to have a mol wt of 81,000. The two preparations were identical in their reactivity towards anti-alpha-acid-glycoprotein. Alpha-acid-glycoprotein markedly shortened the partial thromboplastin time (PTT), but no effect on prothrombin index was noted. At those concentrations which shortened the PTT, the preparation was able partially to reverse the anticoagulant activity of heparin. The possibility that this protein plays a role in the etiology of coagulation disorders in cancer is discussed. (Author abstract) (21 Refs)

15. COLLAGEN CELL ATTACHMENT PROTEIN FROM RAT HEPATOMA CELLS.

Dickey WD, Seals CM, Klebe RJ
Div. Immunology and Allergy, Dept. Pediatrics, Univ. Texas Medical Branch, Galveston, TX, 77550
Cancer Res; 41(10):4027-4030 1981

This paper describes the isolation and partial characterization of a collagen cell attachment protein from the spent culture medium of rat hepatoma cells. When compared with serum fibronectin, this attachment protein differed in several biochemical parameters. The hepatoma attachment protein was partially purified by adsorbing and eluting from an inorganic gel, magnesium oxide. Cell adhesive activity was routinely recovered at levels of 10 to 30%, and a 2,000-fold purification was attained. The hepatoma attachment protein was shown to be sensitive to trypsin and chymotrypsin, to be heat inactivated at 61 C, to have a mol wt of 58,000, to have an isoelectric point of 4.1, to show an electrophoretic mobility on cellulose acetate of approx one-half that of fibronectin, and not to cross-react with antifibronectin antisera. (Author abstract) (27 Refs)

16. URINARY PROTEIN PROFILES IN PATIENTS WITH UROTHELIAL BLADDER TUMOURS.

Hemmingsen L, Rasmussen F, Skaarup P, Wolf H
(c/o Skaarup) Urological Dept. H., Herlev Univ. Hosp., DK-2730 Herlev, Denmark
Br J Urol; 53(4):324-329 1981

Urinary protein profiles were studied in 32 patients with urothelial bladder tumors, using immunonephelometry. In patients with and without residual or recurrent tumors a glomerular type of proteinuria was demonstrated. In patients with Grade III-IV transitional cell carcinoma the glomerular proteinuria was more severe than in patients with Grade I-II tumors. Increased excretion of low mol wt proteins was observed only in patients with Grade III-IV tumors. The use of the increased relative clearance of albumin, transferrin, and haptoglobin as a diagnostic test was attempted in detecting residual or recurrent tumors. In patients with Grade III-IV tumors the predictive value of a positive or negative test was 92% and 100% respectively, whereas the relative protein clearance was without predictive value in patients with Grade I-II tumors. (Author abstract) (31 Refs)

17. STRUCTURAL STUDIES OF A HUMAN GAMMA3 MYELOMA PROTEIN (GOE) THAT BINDS STAPH PROTEIN A.

Recht B, Frangione B, Franklin E, Van Logheim E
Irvington House Inst., Dept. Medicine, New York Univ. Medical Center, 550 First Ave., New York, NY, 10016
J Immunol; 127(3):917-923 1981

The partial amino acid sequence of the Fc region of an unusual monoclonal Ig molecule (Goe), which had the allotypic markers Gm (b0, b3, b5, s, t, v), rarely encountered in Caucasians, was determined. Protein Goe was previously shown to belong to the gamma 3 subclass by antigenic typing, to possess a gamma3-like hinge region and a gamma1-like carboxy-terminal octadecapeptide, and to bind to staphylococcal protein A. The sequence of protein Goe resembled that of gamma3 molecules except for the presence of tyrosine at position 296, alanine at position 339, and histidine and tyrosine at positions 435 and 436. It is of interest that histidine 435 appears to play an important role in binding to Staph protein A. Since tyrosine and phenylalanine at 296 and 300 are typical of G3m(g) molecules, whereas protein Goe is G3m(g-), this may correspond to the non-b1 allotypic marker. Of the numerous explanations to account for these findings, the most likely possibilities are that protein Goe is either a hybrid molecule or the product of a germ line gene representing the G3m s allotype, which is rare in Caucasians and common in Mongoloid populations. Support for the latter alternative is provided by the isolation from normal serum of a small amount of a protein having many of the properties of protein Goe. (Author abstract) (55 Refs)

18. IMMUNE (GAMMA) INTERFERON PRODUCED BY A HUMAN T-LYMPHOBLAST CELL LINE.

Nathan I, Groopman JE, Quan SG, Bersch N, Golde DW
(c/o Golde) Div. Hematology-Oncology, Dept. Medicine, Univ. California Sch. Medicine, Los Angeles, CA, 90024
Nature (Lond); 292(5826):842-844 1981

The characterization of the interferon-gamma (IF-g) produced by a T-lymphocyte cell line (mo) derived from the spleen of a patient with hairy cell leukemia is reported. High IF-g levels were obtained in the presence of fetal calf serum, phytohemagglutinin (PHA), and 12-O-tetradecanoylphorbol-13-acetate; however, in serum-free medium, PHA induction alone gave max IF yield. The Mo IF was of the gamma type by the criteria of acid lability, stimulation of induction, production kinetics, and failure of neutralization by WBC IF antisera. Chromatographic analyses showed that IF-g was a hydrophobic glycoprotein with an apparent mol wt of 40,000. Physiochemically, Mo IF-g was distinct from the erythroid-potentiating a ctivity, colony-stimulating activity, and neutrophil migration-inhibitory factor produced by Mo cells. This cell line should allow the large-scale production of IF-g for purification and clinical trials. (30 Refs)

19. FURTHER STRUCTURAL AND ANTIGENIC STUDIES OF LIGHT-CHAIN AMYLOID PROTEINS.

Natvig JB, Westermark P, Sletten K, Husby G, Michaelsen T
Inst. Immunology and Rheumatology, F. Qvamsgt. 1, Oslo 1, Norway
Scand J Immunol; 14(1):89-94 1981

The major subunit protein of amyloid fibrils (758) isolated from a patient with systemic amyloidosis and studied by N-terminal amino acid sequence analysis was found to be almost identical to the sequence of a VlambdaIV Bence-Jones protein and a previously described AlambdaIV amyloid protein. The AlambdaIV amyloid proteins showed strong antigenic cross-reaction, appearing as antigenic identity in double immunodiffusion tests using anti-AlambdaIV antiserum raised against one or the other of the two proteins. In addition, another new AlambdaV amyloid fibril protein (RS) showed strong amino acid sequence homology and antigenic identify in double immunodiffusions with the prototype of the AlambdaV subgroup (the AR protein). Finally, 20 primary or myeloma-associated amyloid proteins were characterized using antisera against the AA and several Ig light-chain-derived amyloid proteins. (Author abstract) (21 Refs)

20. STRUCTURAL PROPERTIES AND TISSUE DISTRIBUTION OF THE ANTIGEN RECOGNIZED BY THE MONOCLONAL ANTIBODY 653.40S TO HUMAN MELANOMA CELLS.

Natali PG, Imai K, Wilson BS, Bigotti A, Cavaliere R, Pellegrino MA, Ferrone S
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JNCI; 67(3):591-601 1981

The hybridoma 653.40S, constructed with splenocytes from an inbred BALB/c mouse immunized with cultured human melanoma cells, secreted an antibody that had been shown to recognize an antigenic determinant restricted to human melanoma cell lines. The monoclonal antibody (MoAb) 653.40S showed immunoprecipitation of two glycopolypeptides synthesized by melanoma cells, one with the apparent mol wt of 280,000 and the other one with a mol wt larger than 500,000. These two glycopolypeptides were not bridged by disulfide bonds and were peripheral rather than integral to the plasma cell membrane. Comparison of the reactivity of cells of the melanocyte lineage with the MoAb 653.40S and with the MoAb Q5/13 to human Ia-like antigens showed that the former reacted with proliferating melanocytes and melanoma cells, whereas the latter reacted only with melanoma cells. The MoAb 653.40S did not react with a large variety of surgically removed normal and tumor tissues except for some instances of basal cell and squamous cell carcinomas. These results suggested that double staining of pigmented skin lesions with the MoAb 653.40S and with an MoAb to Ia-like antigens may help to solve controversial diagnosis of melanoma. Furthermore, the MoAb 653.40S may be useful for radioimaging and immunotherapy of melanoma. (Author abstract) (32 Refs)

21. A HYPOTHETICAL SPACE-FILLING MODEL OF THE V-REGIONS OF THE GALACTAN-BINDING MYELOMA IMMUNOGLOBULIN J539.

Feldmann RJ, Potter M, Glaudemans CP
Computer Center Branch, Div. Computer Res. and Technology, NCI, Bethesda, MD, 20205
Mol Immunol; 18(8):683-698 1981

J539 is an IgA myeloma protein whose V-region amino acid sequences and ligand binding for beta-1,6-galactosyl oligosaccharides have been determined in the past. Two hypothetical, three-dimensional models of this protein have now been constructed on a computer display system by modifying the known structures of McPC603. The J539 Fv is 11 amino acids shorter than McPC603, and its alpha-carbon backbone was formed by eliminating positions from McPC603 and rejoining the chains at three deletion sites in CDR-1 of VL and CDR-2 and-3 of VH. This maneuver was performed using a three-dimensional viewing system that displayed the amino acids with side chains in the regions around in break sites. The side chains of the amino acids in the model were oriented by a computer program that searched the available space for positions that did not make van der Waals contacts with neighboring atoms. Beta-1,6-Galactohexasoe has been fitted to the J539 combining site using the computer site using the computer display system. The terminal non-reducing sugar made close contacts with hydrogen bond accepting amino acids in CDR-2 of VH and oxygens of the third and fourth sugars were close to hydrogen bond accepting amino acids in CDR-2 of VH and oxygens of the third and fourth sugars were close to hydrogen bond accepting amino acids in CDR-1 and CDR-3 of VL. While the proposed structures are not unique solutions for J539 they present working models for further immunochemical experiments. (Author abstract) (43 Refs)

22. POLYDISPERSITY OF PROTEOGLYCANS SYNTHESIZED BY CHONDROCYTES FROM THE SWARM RAT CHONDROSARCOMA.

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J Biol Chem; 256(15):7883-7889 1981

The population of proteoglycan monomers in aggregates was purified from chondrocyte cultures after labeling with either

[3H]serine and [35S]methionine or [3H]serine and [35S]sulfate. Digestion of the monomers labeled with [3H]serine and [35S]methionine with trypsin indicated that serine was enriched (approx 80%) in the chondroitin sulfate attachment region of the core protein, while methionine was enriched (approx 60%) in the hyaluronic acid-binding region. Sepharose CL-2B chromatography and velocity gradients were used to isolate monomer subfractions which differed in molecular size. The 3H/35S ratios for the subfractions from monomers labeled with [3H]serine and [35S]methionine were nearly constant, indicating that the core protein lengths were constant regardless of the size of the monomer. Conversely, the 3H/35S ratios for subfractions of monomers labeled with [3H]serine and [35S]sulfate increased significantly with decreasing sizes. Chondroitin sulfate chains in subfractions were released by alkaline borohydride treatment. The mean mol wt of the chondroitin sulfate chains decreased from approx 18,500 in the largest subclass of monomers to approx 12,500 in the smallest. [3H]Serine-labeled monomer subfractions were digested with papain to determine the proportion of serine residues substituted with chondroitin sulfate. The distribution of label was constant for greater than 90% of the monomers from the largest to the smallest. The results indicate that, for at least 90% of the newly synthesized monomers that are able to aggregate, variation in chondroitin sulfate chain size is the only contributing factor to polydispersity in their molecular size. (Author abstract) (50 Refs)

23. IDENTIFICATION OF CORE PROTEIN, AN INTERMEDIATE IN PROTEOGLYCAN BIOSYNTHESIS IN CULTURED CHONDROCYTES FROM THE SWARM RAT CHONDROSARCOMA.

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Lab. Biochemistry, Natl. Inst. Dental Res., NIH, Bethesda, MD, 20205
J Biol Chem; 256(15):7890-7897 1981

After incubating cultured chondrocytes from the Swarm rat chondrosarcoma for 30 min with [3H]serine, a labeled macromolecule was found predominantly as Mr = approx 370,000 species which was subsequently identified as a core protein precursor to cartilage proteoglycan from the following properties: (a) it was immunoprecipitated along with completed proteoglycan from cell extracts by an antiserum to the complex of hyaluronic acid-binding region, link protein, and hyaluronic acid. Its immunoprecipitation could be inhibited completely by the addition of purified hyaluronic acid-binding region to the extracts, indicating the presence of common antigenic determinants with this region of the proteoglycan core protein. (b) The core protein precursor was able to interact with the hyaluronic acid and link protein in proteoglycan aggregates added as carrier to extracts to form mixed aggregates of high buoyant density in associative CsCl density gradients. Labeled core protein precursor and link protein were subsequently isolated from the mixed aggregates from the top of dissociative CsCl density gradients. (c) Radioactivity in core protein precursor after a 30-min pulse of [3H]serine disappeared after inhibiting further protein synthesis with cycloheximide concurrent with the appearance of label in completed proteoglycan molecules. (Author abstract) (32 Refs)

24. CLINICAL AND SECRETORY DIFFERENCES IN PANCREATIC CANCER AND CHRONIC PANCREATITIS .

Goodale RL, Condie RM, Gajl-Peczalska K, Taylor T, O'Leary J, Dressel T, Borner JW, Frick MP, Fryd DS
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Ann Surg; 194(2):193-198 1981

The differential diagnosis between chronic pancreatitis and pancreatic cancer can be very difficult. In 60 patients with either of these conditions, who had satisfactory ERCP study, clinical features were correctly matched with the final diagnosis by discriminant analysis in 44 (73%). The sensitivity of ERCP radiographic findings in pancreatic cancer was 80% and sensitivity of cytology was 54%. To see if exocrine function was specific for cancer, fresh pancreatic secretions were aspirated in 27 patients at the time of ERCP. By isoelectric focusing, a pattern of extreme zymogen depletion was observed in chronic alcoholic pancreatitis (Group 1), pancreatic cancer (Group 2), and chronic nonalcoholic pancreatitis (Group 3). The three groups were not distinguishable. By contrast, significant changes in albumin, IgG and IgA concentrations were seen in Group 2. The albumin level was over 10-fold greater than in Groups 1 and 3 (p less than 0.02 and less than 0.05). The IgG was 7-fold and 2-fold greater (p less than 0.01 and greater than 0.2) and the IgA was 15-fold and 6-fold greater (p less than 0.002 and less than 0.05) than in Groups 1 and 3, respectively. The two groups of pancreatitis had similar concentrations of albumin and IgA. The ratio of albumin to IgG was also different in Group 2 from the other groups, suggesting different mechanisms for the appearance of proteins in pancreatic secretions. Nonzymogen protein levels can distinguish chronic pancreatitis from pancreatic cancer, and further study of them may identify useful tumor-specific markers. (Author abstract) (31 Refs)

BINDING/ RECEPTOR PROTEINS

25. GLUCOCORTICOID RECEPTOR DETERMINATION IN LEUKEMIA PATIENTS USING CYTOSOL AND WHOLE-CELL ASSAYS.

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Cancer Res; 41(10):3979-3984 1981

Parallel determinations of glucocorticoid receptors in the cells of patients with various forms of leukemia were made by two assay methods, one using cell-free cytosolic extracts and the other using whole-cell preparations. Both assays revealed saturable binding of triamcinolone acetonide in all cases. The mean equilibrium dissociation constant for the interaction of triamcinolone acetonide with the cytoplasmic receptor at 2 C was 9.45 +-6.33 (S.D.) nM while that for the whole-cell binding at 37 C was 6.13 +-3.25 nM, suggesting an increase in receptor affinity at physiological temperatures. Competition experiments with various unlabeled steroids revealed a higher degree of glucocorticoid specificity at 37 C in whole-cell suspensions than at 2 C in cytosol. In a comparative analysis of 41 leukemic cell specimens, it was found that determinations carried out by whole-cell assay, calculated as number of sites per cell, correlated well with those performed by cytosol assay, calculated as fmol/mg protein, independent of the type of leukemia. However, for cells with low receptor content, the two assay methods were more difficult to compare. In agreement with previous reports, the cytosol assay consistently underestimated the number of receptors with respect to the whole-cell assay, particularly in cases of lymphatic leukemia. Furthermore, the underestimation decreased for increasing levels of total cellular receptor. These results suggest that, in addition to possible defects in the cytoplasm-to-nucleus translocation process, the acceptor-binding capacity of the nucleus may also represent one of the factors which determines the levels of assayable cytoplasmic receptors. Moreover, they indicate that the two assay methods furnish nonequivalent information. (Author abstract) (28 Refs)

26. MONOCLONAL ANTIBODY OKT-9 RECOGNIZES THE RECEPTOR FOR TRANSFERRIN ON HUMAN ACUTE LYMPHOCYTIC LEUKEMIA CELLS.

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J Immunol; 127(3):1256-1258 1981

The monoclonal antibody OKT-9 recognizes a surface protein of human lymphocytes that consists of a disulfide bonded homodimer of mol wt 200,000 intact and 95,000 reduced. A similar protein is precipitated by transferrin-agarose, but not by agarose alone. Peptide mapping by limited proteolysis shows that the proteins precipitated by OKT-9 antibodies and transferrin-agarose are homologous. It is concluded that OKT-9 antibodies recognize the transferrin receptor. Expression of receptors for transferrin may be useful as a marker for activated of dividing cells. (Author abstract) (21 Refs)

MEMBRANE/ SURFACE PROTEINS

27. BIOCHEMICAL ANALYSIS OF NORMAL HUMAN TISSUES AND THOSE OF LYMPHOMA PATIENTS BY HIGH-RESOLUTION PROTEIN MAPPING.

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J Cancer Res Clin Oncol; 101(1):43-51 1981

Membrane proteins from various normal human tissues, (tonsils, prostate, etc) and from tissues of lymphoma patients (spleen and lymph nodes) were mapped by high-resolution, two-dimensional electrophoresis. The protein maps showed high tissue specificity for normal tissues, and they were sensitive to pathologic deviations from normal. Changes found in the protein maps of patients with hairy cell leukemia, chronic lymphatic leukemia, immunocytoma, plasmacytoma, and centroblastic centrocytic lymphoma are described. The pattern of proteins from different lymphomas differed more from each other than those of normal tissues, a factor that might be useful in the development of a 'tumor-specific protein code.' The code could be represented by the spatial distribution and relative intensities of proteins stained with coommassie brilliant blue or, in the case of minor proteins, a sensitive silver stain. A protein code would provide the pathologist with an additional set of parameters for tumor identification, characterization, and classification. (11 Refs)

28. DISTRIBUTION AND MOLECULAR CHARACTERIZATION OF A CELL-SURFACE AND A CYTOPLASMIC ANTIGEN DETECTABLE IN HUMAN MELANOMA CELLS WITH MONOCLONAL ANTIBODIES.

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Int J Cancer; 28(3):293-300 1981

The monoclonal antibodies 225.28S and 465.12 to human melanoma-associated antigens have been tested with a large variety of surgically removed skin lesions and malignant tumors as well as with a panel of cultured cell lines in serological and immunochemical assays. The antibody 225.28S reacts with a plasma membrane antigen while the antibody 465.12 detects a cytoplasmic antigen. Both antibodies fail to react with melanocytes from normal skin as well as benign skin lesions but react with nevi, melanoma cells and some skin carcinomas. Analysis with surgically removed tumors and cultured human cell lines indicated that the plasma membrane antigen is restricted to skin lesions whereas the cytoplasmic antigen is synthesized by tumor cells of various histological origins. The plasma membrane antigen is composed of two glyco-polypeptides of 280,000 and greater than 440,000 daltons, while the cytoplasmic antigen consists of four glycopolypeptides of 94,000, 75,000, 70,000 and 25,000 daltons. None of these components are bridged by disulfide bonds. The cytoplasmic antigen was readily detected in the spent culture medium of melanoma cell lines in the form of a major 94,000 dalton and a minor 72,000 dalton structure, while the plasma membrane antigen was detectable only after vastly increasing the sensitivity of the assay system. (Author abstract) (23 Refs)

29. MOLECULAR WEIGHT ANALYSIS OF FCGAMMA-BINDING PROTEINS OF LYMPHOID LEUKEMIA, MYELOID LEUKEMIA, AND HAIRY-CELL LEUKEMIA.

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J Cancer Res Clin Oncol; 101(1):75-80 1981

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) previously indicated that a membrane protein isolated from the EDTA-mercaptoethanol lysates of B-cell lymphocytic leu kemias had an apparent mol wt of 28,000 daltons. The protein bound to the Fc-region of IgG molecules. SDS-PAGE analysis of Fcgamma-binding proteins from two patients with chronic myeloid leukemia revealed a 42,000-and an 18,000-mol-wt protein. The Fcgamma-binding material from two hairy cell leukemias electrophoresed in the form of a closely spaced double peak in the 28,000-mol-wt region. Subsequent autoradiographic SDS slab gel electrophoresis revealed that one component of the double peak had a mol wt of 28,000 daltons and corresponded to the Fcgamma-binding material of the B-type lymphocytic leukemia cells. The second component had a lower mol wt and was not present on lymphoid or myeloid cells. These findings argue against a relationship between hairy cells and myeloid cells and in favor of a relationship between hairy cells and B cells. (16 Refs)

30. METASTATIC POTENTIAL CORRELATES WITH CELL-SURFACE PROTEIN ALTERATIONS IN B16 MELANOMA VARIANTS.

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Nature; 293(5827):74-76 1981

Mice cell lines of differing metastatic potential in an in vivo assay (im injection into 9-12-wk-old C57BL mice of 2.5 x 10(4) cells from subconfluent cultures) were studied: B16-F10Lr cells (less than 5% pulmonary metastasis), B16-F10 cells (25-33% multiple lung metastases), and B16-BL6 (60-75% pulmonary metastasis); F10 cells were derived from F10Lr cells by successive in vivo/in vitro passaging, and BL6 cells were derived from F10 cells by selection for increased invasive capacity. Treatment with 1 M urea following enzymatic pre-iodination of cell-surface proteins and subsequent electrophoretic analysis showed low mol wt (approx 18,000 [18 K]) and high mol wt (approx 78 K) components from F10Lr cells, but only the high mol wt components from F10 cells. However, 8 M urea extracts of pre-iodinated F10Lr cells gave lower levels of the fast-migrating (18 K) protein, did not reveal the presence of several fast-migrating components, and showed the presence of slower-migrating components; this result may be due to the release or activation of a protease from the nonmetastatic cells by 1 M urea that may be inhibited by 8 M urea. Electrophoresis following the promotion of membrane vesiculation on surface proteins with p-formaldehyde and dithiothreitol revealed similar patterns in the two metastatic cell lines but a different pattern in the low-metastasis line; the latter showed an 18 K species, the absence of a 45 K species, and the presence of a significant species that migrated slightly faster than the 78 K species of the metastatic lines. Clones of the three cell lines at limiting dilutions revealed that the demonstrated differences were retained in the clones. Extraction of surface-iodinated cells with Triton-X followed by electrophoresis, revealed that the Triton-insoluble cell-surface components of the metastatic cell lines gave similar patterns with comparable amount of the 78 K species, the low-metastasis cell line had a greater level of radioactivity in the fast-migrating species. This is apparently the first report of cell-surface protein differences between cells of different metastatic potential. (16 Refs)

31. SIALIC ACID METABOLISM IN REGENERATING RAT LIVER.

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Cancer Lett; 13(4):281-289 1981

Sialic acid content and levels of the enzymes responsible for activation, transfer, and hydrolysis of sialic acids in the livers of control, sham-operated, and two-thirds-hepatectomized male rats were studied. At 24 and 48 hr after surgery, the specific activities of cytidine monophosphate-[4,5,6,7,8,9-14C] N-acetylneuraminic acid (CMP-NANA) synthetase and sialyltransferase in regenerating liver, as well as in livers of sham-operated rats, were significantly higher than in control liver tissues removed at surgery. Combined range of values for the two enzymes (in nanomole/mg protein) were 401-452 vs 324 control and 0.47-0.57 vs 0.36 control, respectively. For each enzyme, values for regenerating and sham-operated liver were not significantly different. Membrane-bound neuraminidase activity was 2x control level in regenerating liver at 24 and 48 hr, but not significantly different from control in sham tissue. Cytosolic neuraminidase activity was similar in all tissue samples. Regenerating liver sialic acid content, determined by thiobarbituric acid assay, was lower at 24 but not at 48 hr than in sham or control liver samples; the changes were observed in whole liver homogenate and in the sedimentable fraction but not in the soluble fraction. Results for CMP-NANA synthetase and sialyltransferase did not constitute changes specifically associated with the regenerating process, since similar changes were seen in regenerating and sham-operated tissue. The selective increase in membrane-bound neuraminidase and decrease in sialic acid content in regenerating liver were of particular interest, in light of reduced activity observed for other regenerating rat liver acid hydrolases and increases in sialic acid seen in various malignant hepatic tissues. (27 Refs)

32. ENDOGENOUS PROTEIN PHOSPHORYLATION IN ISOLATED PLASMA MEMBRANES OF AH-66 HEPATOMA ASCITES CELLS.

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J Biochem (Tokyo); 90(1):23-31 1981

The phosphorylation of endogenous membrane proteins by an endogenous protein kinase was studied in isolated plasma membranes from AH-66 hepatoma ascites cells using [gamma-32P]ATP as a pre cursor. The phosphorylation occurred very rapidly in the presence of 10 mM Mg++ and reached a max level at 2 min. Ca++ strongly inhibited the phosphorylation reaction and antagonized the activation produced by Mg++. Neither cyclic AMP nor cyclic GMP had a significant effect on the phosphorylation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography showed that only membrane proteins ranging in mol wt from 125,000 to 200,000 were heavily phosphorylated and those of less than 60,000 mol wt were not phosphorylated at all. The protein kinase activity was readily extractable from the plasma membranes with 1 mM EDTA at pH 8.5. Among exogenous substrates, the extracted protein kinase catalyzed the phosphorylation of histone, protamine and phosvitin rather than casein. When the extracted protein kinase was subjected to chromatography on DEAE-Sepharose, a single major peak of cyclic AMP-independent protein kinase was eluted at a position quite different from those of the cytosolic protein kinases. (Author abstract) (23 Refs)

33. ANODIC MIGRATION OF ACTIN AND INCREASED LEVELS OF A CATHODIC FIBRONECTIN AMONG THE CYTOSKELETAL CHANGES THAT CORRELATE WITH INCREASED CELL ADHERENCE IN LIVER EPITHELIOID TUMOR CELLS.

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Cell Biol Int Rep; 5(8):791-796 1981

Molecular alterations in cytoskeletal organization were examined in R72/3 liver epithelioid tumor cells which become more adherent to their growth substratum upon exposure to the halogenated pyrimidine iododeoxyuridine (IUDR). Cells were grown for 72 hr in the presence of 2.5 ug/ml IUDR, then extracted successively with 1% and 0.5% Triton-X-100 detergent. Once IUDR had been shown to promote cell adherence (in EDTA-treated cultures, 37% versus 73% of control cells were detached; without EDTA 2.5% versus 4% of controls were detached), simultaneous two-dimensional analysis was performed on samples solubilized in 8 M urea. Isoelectric focusing with Ampholines in the pH range 3-10 followed by polyacrylamide gel co-electrophoresis in the same slab showed clear differences with respect to non-IUDR-exposed controls. Seen were an increase in the expression of a 220,000 (220K) dalton component towards the cathode, only an anodic 64K dalton species, a decrease in the more anodic of three species appearing in the 50K dalton region, and most actin-like 44K dalton components migrating closer to the anode. Assay for fibronectin in the two-dimensional gel revealed that the 230K component detected in IUDR-treated cells is antigenically related. The data suggest an involvement of increased fibronectin levels in the adherence observed in IUDR-treated cells. The presence of both fibronectin- and actin-like molecules among the cytoskeletal components is confirmed. The results indicating that levels of a cathodic fibronectin increase under conditions in which actin becomes more anodic suggest that the two macromolecules may interact in an electrostatic manner across the plasma membrane in vivo. (16 Refs)

34. LIVER PLASMA MEMBRANES AND PROTEOGLYCAN PREPARED THEREFROM INHIBIT THE GROWTH OF HEPATOMA CELLS IN VITRO.

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Biochim Biophys Acta; 646(1):161-168 1981

Plasma membranes from rat liver or kidney inhibited the growth of hepatoma (AH-130) cells in vitro. AH-130 plasma membranes or RBC ghosts inhibited the growth of AH-130 cells less effectively. The inhibitory activity of liver plasma membranes was lost by heat treatment, or mild protease (papain or bromelin, but not trypsin or pronase) treatment, whereas it was retained after sialidase treatment or delipidation by ethanol/ether. Proteoglycan (proteoheparin sulfate) prepared from liver plasma membranes inhibited the growth of AH-130 cells, but heparin sulfate was less active. The inhibitory activity of liver plasma membranes seemed, however, not to be ascribable solely to proteoheparin sulfate associated with plasma membranes. Preliminary investigations suggested that the mol wt 40,000 component may be a major inhibitory principle in liver plasma membranes. (Author abstract) (18 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

35. DIFFERENCE IN GENETIC EXPRESSION BETWEEN NORMAL AND TRANSFORMED WI-38 CELLS.

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Cell Struct Funct; 6(2):93-101 1981

In order to study transformation-specific gene expression, total poly(A)+RNA was extracted from normal WI-38 and Co60-irradiation-transformed WI-38 (CT-1) human diploid fibroblasts, complementary DNA (cDNA) against this RNA was prepared, and hybridization studies of these two nucleic acids were performed; also, radiolabeled proteins from the normal and transformed cells were prepared and examined by two-dimensional gel electrophoresis. Poly(A)+RNA sedimenting from 5S to 23S was hybridized to cDNA fractions greater than 150 bases (av size: 300 bases) in two combinations: homologous (both nucleic acids came from the same cell type) and heterologous (the RNA and DNA came from opposite cell types); considerable hybridization occurred in both cases, showing that there is extensive homology between the poly(A)+RNAs of normal and transformed cells. Approx 200 protein spots were examined electrophoretically from both cell types, first by isoelectrofocusing and then by sodium dodecyl sulfate-polyacrylamide gel analysis followed by autoradiography: two proteins were found in the normal cells that were not present in the transformed cells, and two proteins were found in the transformed cells that were not found in the normal cells. These results indicate that transformation of human fibroblasts is accompanied by only limited alteration in genetic expression. (25 Refs)

36. IMMUNOCYTOCHEMICAL DEMONSTRATION OF INTRACYTOPLASMIC ALKALINE PHOSPHATASE IN HELA TCRC-1 CELLS.

Tokumitsu S, Tokumitsu K, Fishman WH
La Jolla Cancer Res. Foundation, La Jolla, CA, 92038
J Histochem Cytochem; 29(9):1080-1087 1981

The ultrastructural localization of alkaline phosphatase has been examined in cells of a HeLa subline (TCRC-1) that are monophenotypic for Regan isoenzyme expression. Enzyme activity was demonstrated at the cell surface plasma membrane and in certain lysosomes as revealed by the lead citrate method. The regular direct immunoperoxidase procedure utilizing antibodies in IgG or Fab' form showed the same distribution patterns of alkaline phosphatase. However, when the cell surface antigen was blocked in advance with specific unlabeled antibodies and direct immunocytochemistry performed in the presence of saponin, intracellular alkaline phosphatase antigen was observed in the perinuclear space, endoplasmic reticulum, and Golgi apparatus. The results appeared to be concordant with the current concept that membrane glycoproteins are formed in the endoplasmic reticulum, modified in the Golgi apparatus and then transported to the cell surface. Intracellular alkaline phosphatase was observed predominantly in some cell populations especially mitotic cells, suggesting that the enzyme protein was synthesized in and around the mitotic phase. Accordingly, this technique of differential membrane immunocytochemistry appears to provide an opportunity to follow ectopic gene expression as a function of cell cycle and enzyme induction. (Author abstract) (28 Refs)

37. WHOLE BODY PROTEIN SYNTHESIS AND TURNOVER IN NORMAL MAN AND MALNOURISHED PATIENTS WITH AND WITHOUT KNOWN CANCER.

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Ann Surg; 194(2):123-128 1981

Plateau enrichment of 15N-ammonia following 24 hr continuous iv infusion of 15N-glycine was used to measure total body protein turnover and synthesis in normal volunteers and malnourished patients, with and without cancer. The mean postabsorptive total body protein synthesis rate in three normal controls was 2.5 g protein/kg/day. Protein synthesis and turnover decreased by a mean of 23% following 1 wk of fasting, and returned to baseline levels following 1 wk of refeeding. In three malnourished patients without known tumor, whole body protein synthesis and turnover was similar to controls; following 7 to 10 days of total parenteral nutrition (TPN) in the non-tumor-bearing patients whole body protein synthesis and turnover decreased by 18%. Three of seven malnourished patients with known tumors had marked elevations in total body protein synthesis and turnover; TPN increased protein turnover in five of seven patients with known cancer. This study suggests that some malignant tumors can increase whole body protein synthesis and turnover in both the malnourished and fed state. This increase in protein turnover may represent a direct effect of the tumor, or reflect concomitant illness. (Author abstract) (24 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

38. BLEOMYCIN-INDUCED PULMONARY FIBROSIS: CORRELATION OF BIOCHEMICAL, PHYSIOLOGICAL, AND HISTOLOGICAL CHANGES.

Hesterberg TW, Gerriets JE, Reiser KM, Jackson AC, CrossCE, Last JA
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Toxicol Appl Pharmacol; 60(2):360-367 1981

Several parameters of pulmonary fibrosis induced by bleomycin (B) were studied. Male Sprague Dawley rats were given B (0.38, 0.75 or 1.5 units) intratracheally. B-treated rats weighed significantly less than controls 1 wk after treatment; after 2 wk only those given the highest dose were below controls wt. After 3 wk all groups weighed the same as controls. Lung wt was increased in all B-treated rats, but not in a dose-related manner. Protein biosynthesis was raised in all groups in a dose-related manner after 1 wk, returning to normal by wk 2 in the high-dose group, but remaining raised in the two lower-dose groups. Collagen synthesis in lung minces was raised by B in a dose-response manner. Collagen content of the lungs was raised at wk 2 and 3 and remained raised 5 and 8 wk after treatment. Histologically lung tissue showed inflammation and hypercellularity at 1 and 2 wk, with increased interstitial collagen at 3 wk. Compliance of lung tissue was reduced at wk 2 and 3 and returned to normal at wk 5 and 8. The results suggest that B-induced fibrosis may be described as either self-limiting or chronic, depending on the parameters used to measure it. Biochemical assays did not correlate with physiological changes 5 and 8 wk after treatment. (26 Refs)

39. REGULATION OF GENE EXPRESSION BY TUMOR PROMOTERS. II. CONTROL OF CELL SHAPE AND DEVELOPMENTAL PROGRAMS FOR MACROPHAGES AND GRANULOCYTES IN HUMAN MYELOID LEUKEMIC CELLS.

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Int J Cancer; 28(3):285-291 1981

Regulation of the developmental programs for macrophages and granulocytes has been analyzed, using two-dimensional gel electrophoresis of the cytoplasmic protein changes, in a human myeloid leukemic cell line (HL60) that can be induced to differentiate to macrophages by the macrophage and granulocyte-inducing (MGI) protein and the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA), and to granulocytes by dimethylsulfoxide (DMSO). Studies on the protein changes induced by the different inducers showed a similar developmental program for macrophage differentiation induced by MGI or TPA, which differed from the beginning from the granulocyte program induced by DMSO. Comparison with normal cells from human peripheral blood has shown that the developmental programs induced in the HL60 cells are relevant to the normal programs of differentiation for these two cell types. Unlike MGI and DMSO, TPA induces rapid attachment of the HL60 cells to the surface of the Petri dish. Combined treatment with TPA and DMSO showed cell attachment, extensive spreading of the cells, the regulation of specific proteins and expression of the macrophage program. The addition of TPA to cells induced for the granulocyte program by DMSO resulted in cell attachment and spreading and a switch-over from the granulocyte to the macrophage program. The results indicate that cells in suspension can express either the macrophage or granulocyte program depending on the inducer, and that changes in cell shape associated with cell attachment induced by TPA can regulate specific proteins and restrict the developmental program to macrophages. It is suggested that the in vivo environment of cells in relation to the possibilities for cell adhesion, which can be regulated by tumor promotors, may play a major role in determining the developmental program of myeloid and other cell types. (Author abstract) (41 Refs)

40. ASSOCIATION OF PHENOTYPIC REVERSION OF TRANSFORMED CELLS INDUCED BY INTERFERON WITH MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE CYTOSKELETON.

Brouty-Boye D, Cheng YS, Chen LB
Lab. Viral Oncology, Institut de Recherches Scientifiques sur le Cancer, BP 8, 94802 Villejuif Cedex, France
Cancer Res; 41(10):4174-4184 1981

The distribution of microfilament bundles, intermediate filaments, microtubules, vinculin plaques, and extracellular fibronectin matrices was examined by indirect immunofluorescence in x-ray-transformed C3H/10T1/2 cells. The transformed phenotype correlated with a lack of actin cables and vinculin-containing plaques. Both interferon-treated and untreated transformed cells displayed a similar distribution of mitochondria, intermediate filaments, microtubules, and fibronectin. Significant qualitative and quantitative differences between the two types of cells were found in the proteins associated with the cytoskeletal framework by two-dimensional gel analysis. (Author abstract) (27 Refs)

41. LUTROPIN-DEPENDENT PROTEIN PHOSPHORYLATION AND STEROIDOGENESIS IN RAT TUMOUR LEYDIG CELLS.

Bakker GH, Hoogerbrugge JW, Rommerts FF, Van der Molen HJ
Dept. Biochemistry, Div. Chemical Endocrinology, Medical Faculty, Erasmus Univ., Rotterdam, The Netherlands
Biochem J; 198(2):339-346 1981

Tumor Leydig cells have been incubated in the presence or absence of luteinizing hormone (lutropin). Stimulation of cells with lutropin (1,000 ng/ml) in the presence of 1-methyl-3-isobutylxanthine (0.25 mM) resulted in increased steroid production and increased protein phosphorylation. When pregnenolone metabolism was inhibited, basal pregnenolone production was 26.9 +-7.4 ng/60 min per 10(6) cells: stimulated production was 156.1 +-39.5 ng/60 min per 10(6) cells (means +-SD, n = 4). Lutropin-dependent phosphorylated proteins of molecular mass 17,000, 22,000, 24,000, 33,000 and 57,000 Da were detected. A significant increase of [32P]Pi incorporation into these phosphorylated proteins was observed concomitant with the increased pregnenolone production. The occurrence of the phosphoproteins in nuclei, mitochondria and postmitochondrial-supernatant was investigated. The 17,000 Da phosphoprotein was found in the nuclear fraction, whereas the 22,000, 24,000, 33,000 and 57,000 Da phosphoproteins were localized in the postmitochondrial-supernatant fraction. Of the cholesterol-side-chain-cleavage activity, 80.3 +-6.1% (mean +- SD, n = 5) was present in the mitochondrial fraction isolated from tumor Leydig cells, and this activity was 2.5-fold increased when cells had been preincubated with lutropin/1-methyl-3-isobutylxanthine (basal production: 194.6 +-28.6 ng/30 min per mg of protein; lutropin-stimulated production: 498.8 +-91.5 ng/30 min per mg of protein; means +- SD, n = 3). The similarities in the kinetics of the phosphorylation of proteins and the pregnenolone production after addition of lutropin/1-methyl-3-isobutylxanthine indicate that the phosphoproteins could be involved in the lutropin-dependent increase in steroidogenesis in tumor Leydig cells. It remains to be demonstrated, however, to what extent the phosphoproteins outside the mitochondria can influence the cholesterol-side-chain-cleavage activity inside the mitochondria. (Author abstract) (31 Refs)

42. SPECIFIC LABELLING OF MICROSOMAL PROTEINS BY REACTIVE INTERMEDIATES GENERATED FROM 2-ACETYLAMINOFLUORENE IN VITRO.

Kaderbhai MA, Bradshaw TK, Freedman RB
Biological Lab., Univ. Kent, Canterbury, CT2 7NJ Kent, England
Chem Biol Interact; 36(2):211-227 1981

Incubation of 2-[9-14C]acetylaminofluorene (2-[9-14C]AAF) in vitro with rat liver microsomes, leads to covalent binding of label to microsomal proteins. The binding is NADPH-dependent, increases linearly with time, and is inhibited by SKF-525A and 7,8-benzoflavone (7,8-BF). Binding is increased more than eight-fold in microsomes from 3-methylcholanthrene (MC)-pretreated rats, but only less than two-fold in those from phenobarbital (PB)-pretreated rats. In the presence of cytosolic proteins, there is slight enhancement of the labeling of microsomes and some labeling of the cytosolic proteins. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional gel electrophoresis indicate that covalent labeling by 2-AAF derivatives is concentrated in specific proteins. The pattern of labelling varies between microsomes from animals pretreated with PB, MC and 2-AAF. Factors which may contribute to the specificity of labeling are discussed. (Author abstract) (21 Refs)

43. RETINOID INHIBITS KERATIN FORMATION OF PIG SKIN EXPLANTS.

Aoyagi T, Kamigaki K, Saruta M, Iizuka H, Miura Y
Dept. Dermatology, Hokkaido Univ. Sch. Medicine, N-15, W-7, Sapporo 060, Japan
J Dermatol (Tokyo); 8(3):207-213 1981

The effect of retinoid on electrophoretic patterns of keratin proteins extracted from epidermal cells cultured in the presence or absence of retinoid was investigated. The highest wt molecular band found in keratin proteins from whole pig epidermis was not found in those from outgrowths grown in vitro. In explants treated with Ro 10-1670, the electrophoretic pattern was significantly altered compared to that of controls. The 63,000 dalton protein band was almost absent in the retinoid-treated explants and the 59,000 dalton protein band was split into two minor bands (mol wt 59,000 and 58,000). It is supposed that the alteration of polypeptide pattern round in retinoid-treated explants indicates reduced keratin formation. (14 Refs)

44. EFFECTS OF THE TUMOR PROMOTER 12-O-TETRADECANOYL-PHORBOL-13-ACETATE ON NEWLY SYNTHESIZED PROTEINS IN MOUSE EPIDERMIS.

Laskin JD, Mufson RA, Piccinini L, Engelhardt DL, Weinstein IB
Div. Environmental Sciences Cancer Center/Inst. Cancer Res., Coll. Physicians and Surgeons, Columbia Univ., 701 West 168th St., New York, NY, 10032
Cell; 25(2):441-449 1981

We have analyzed the effects of treatment of mouse epidermis with the potent tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate on the profile of newly synthesized proteins. TPA was applied to the skin of the intact mouse, and either 3 or 24 hr later skin fragments were pulse-labeled in vitro with 35S-methionine for 4 hr. The epidermal proteins were extracted and separated by two-dimensional gel electrophoresis. Over 200 individual proteins were resolved in acidic gels. At least 10 of these showed major (by a factor of 5 or more) increases or decreases in response to TPA; 8 of these appear to be keratin proteins. Two-dimensional gel profiles of basic proteins synthesized by mouse epidermis resolved over 100 individual proteins. Only one of these showed a significant change in response to TPA. This 41 kd protein increased more than 100-fold within 24 hr after the application of TPA. Treatment of mouse skin with mezerein, a plant diterpene structurally related to TPA, produces an almost identical change in the pattern of proteins produced. Four agents that induce hyperplasia but are not potent tum or promoters, ethylphenylpropiolate, acetic acid, turpentine oil and the Ca++ ionophore A23187, modulate the synthesis of only three of the keratin proteins. Thus the changes in protein profiles induced by TPA and mezerein are not simply the consequence of hyperplasia. In addition, application to mouse skin of a glucocorticoid that is a potent inhibitor of tumor promotion inhibits most of the changes in protein profiles induced by TPA. Taken together, these results indicate that TPA and mezerein induce early and marked changes in the profile of specific epidermal proteins. It seems likely that some of these changes are directly related to the process of tumor promotion. (Author abstract) (39 Refs)

DIETARY AND NUTRITIONAL EFFECTS

45. EFFECT OF PARENTERAL NUTRITION ON PROTEIN SYNTHESIS IN ADULT CANCER PATIENTS.

Stein TP, Buzby GP, Rosato EF, Mullen JL
Surgical Res. Lab., Graduate Hosp., Univ. Pennsylvania, Philadelphia, PA, 19146
Am J Clin Nutr; 34(8):1484-1488 1981

The rate of whole body protein synthesis was measured with 15N glycine in three groups of subjects: (1) normal healthy adult controls, (2) semistarved adult patients with upper gastrointestinal tract malignancies and, (3) a group of patients similar to (2) except they were maintained parenterally on a regimen adequate in amino acids and glucose. By comparing the patient results with data obtained from rat studies with 15N glycine we concluded that the patients were protein rather than energy depleted. (Author abstract) (21 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

46. POLYAMINE RESPONSES IN A SOLID TRANSPLANTED TUMOR (S180) IN LIVER AND IN URINE DURING ENDOTOXIN-INDUCED TUMOR INJURY IN THE MOUSE.

Roth H, Kitta D, Jones GR, Osswald H, Kunz W
Inst. Biochemistry, German Cancer Res. Center (05 DKFZ), Im Neuenheimer Feld 280, D-6900 Heidelberg 1, W. Germany
Cancer; 48(4):945-950 1981

Polyamine (PA) and ATP concentrations have been measured in the S180 sarcoma damaged by endotoxin (1) 4 hr after administration, coinciding with the onset of hemorrhage, (2) at 8 hr, and (3) at 24 hr, preceding overt necrosis. The putrescine (PU) content increased promptly, the spermidine (SPD) level dropped by 30% between 8 and 24 hr, while the spermine (SPM) concentration remained unchanged. The ATP level fell, reaching 1% of the control value at 24 hr. The parallel between increases in tumor PU content and urinary PU excretion appears to be largely fortuitous; no similar correlations existed in the cases of SPD and SPM. The development of tumor stasis and the alterations seen in PA metabolism of tumor-free controls suggest that the changes in liver PA concentrations and in urinary excretion primarily reflect responses of nonmalignant cells. Both these findings in animals without tumors and existing data from other experiment al systems are consistent with the thesis that PA response patterns to a particular procedure are qualitatively similar regardless of the presence or absence of a malignant growth, and are characteristic of the procedure employed. (Author abstract) (27 Refs)

OTHER RELATED STUDIES

47. GROWTH OF HUMAN MAMMARY EPITHELIAL CELLS ON COLLAGEN GEL SURFACES.

Yang NS, Kube D, Park C, Furmanski P
Dept. Biology, Michigan Cancer Foundation, Detroit, MI, 48201
Cancer Res; 41(10):4093-4100 1981

We have developed a method for sustained growth of human mammary epithelial cells in monolayer cultures. Epithelial organoids derived from solid breast tissues were grown on the surface of thin (approx 1 mm) collagen gel layers in an enriched growth medium supplemented with hormones, growth factors, fetal calf serum, and horse serum. To transfer the cultures, the collagen layers were dislodged and digested with collagenase. The monolayers of cells released into suspension were then dissociated into single cells using trypsin-ethylenediaminetetraacetate. Dissociated single cells were repleted with 75 to 95% efficiency onto collagen layers or tissue culture plastic surfaces. The dissociated cells could also be cryopreserved and reactivated with greater than 80% plating efficiency on collagen layers. Normal human mammary epithelial cells grown under these conditions progressed through 12 to 15 population doublings. The population-doubling times for normal and malignant mammary cells on collagen layers were 34 and 65 hr, respectively. After reaching confluence, cells in some cultures, derived from either normal or malignant tissues, penetrated the gel surface and grew into the collagen. Within the gels, the cells became organized into three-dimensional tubular structures. The use of collagen layers eliminates a major problem in growth of human mammary epithelial cells in culture, difficulty in efficient dissociation, and cell transfer from monolayers. (Author abstract) (11 Refs)

48. FURTHER STUDIES ON THE CYTOCHEMISTRY OF THE STANDARDIZED SILVER STAINING OF INTERPHASE NUCLEOLI IN SMEAR PREPARATIONS OF YOSHIDA ASCITIC SARCOMA CELLS IN RATS.

Likovsky Z, Smetana K
Inst. Experimental Medicine, Czechoslovak Acad. Sciences, Albertov 4, CS-128 00 Praha 2, Czechoslovakia
Histochemistry; 72(2):301-313 1981

The modification and standardization of simple silver staining procedures for the visualization of the protein component characteristic for silver stained granules (SSG) in interphase nuclei of Yoshida ascitic sarcoma cells is reported. A one-step procedure for silver staining for selective demonstration of nucleolar SSG and a two-step procedure for silver staining for the simultaneous demonstration of nucleolar SSG and silver stained matrix (SSM) are described. The success of the procedures depende d mainly on the quality of silver nitrate and formaldehyde. When both chemicals were standardized, the one- and two-step procedures provided standard results disregarding the origin and batch of silver nitrate or formaldehyde. The silver staining could be easily modified either for the selective demonstration of nucleolar SSG or for the simultaneous demonstration of both SSG and SSM. While the proteins of SSG and SSM seem to be different, both belonged to the group of acid non-histone proteins. Digestion experiments demonstrated the possibility that the acidic proteins of SSG may also be associated with DNA. Biological experiments demonstrated that the number of SSG in Yoshida ascitic sarcoma cells decreases after treatment of the animal with actinomycin D and therefore depends on the state of the nucleolar RNA synthesis. (30 Refs)

49. EFFECT OF DIETARY AMINO ACIDS ON TUMOUR GROWTH AND CELL-MEDIATED IMMUNE RESPONSES.

Bounous G, Sadarangani C, Pang KC, Kongshavn PA
Centre Hospitalier Universitaire, Sherbrooke PQ J1H 5N4, Canada
Clin Invest Med; 4(2):109-115 1981

The effects of various diets were studied in C3H/NCrlBR, C57Bl/6, and (AxC57Bl/6)F1 mice. Diet 1, the standard defined-formula amino acid diet, contained about the same amino acid distribution as casein. Diet 2 contained reduced amounts of l-methionine, l-phenylalanine, and l-tyrosine, and no cystine. Diet 3 contained almost double the amount of amino acids found in diet 1. The Puria mouse chow used contained approx 18% protein. Diet 1 supported normal growth in C3H/NCrlBR mice. Diet 3 and Purina did not produce more body growth than diet 1. Diet 2 produced impaired body growth and reduced spleen wt and WBC counts. Skin grafts were rejected sooner on mice fed diet 2 than on mice fed diet 1. Mice fed diet 2 had higher resistance to Listeria monocytogenes infection than those fed diet 1 or Purina. Diet did not affect the time of appearance of palpable fibrosarcomas. Palpable mammary adenocarcinomas appeared soonest in mice fed diet 3 or Purina. Diets 1 and 2 had about the same inhibitory effect on fibrosarcoma growth. Diet 2 had a greater inhibitory effect on the growth of mammary adenocarcinomas. Tumor growth showed a direct dose-response relationship to amino acid content of the diet. Graft-vs-host reactivity was not affected by diet. Evidently reduction of amino acid inhibits tumor growth, while excessive amino acids promotes tumor growth. This suggests that current concepts of 'good nutrition' promoting immune defenses may be in error. (26 Refs)

50. CHANGES IN TYROSINE LEVELS OF BLACK AND WHITE SKINS OF GUINEAPIGS TREATED WITH PSORALEN AND HORMONES.

Mondal M, Banerjee PK

Dept. Physiology, Univ. Coll. Medicine, Calcutta Univ., Calcutta 700 009, India

Indian J Exp Biol; 19(2):189-190 1981

Adult guinea pigs of each sex received no treatment, 1 mg psoralen, 0.24 mg testosterone propionate injected ip in males every other day for 5 days, 7.5 mg hydrocortisone ip, 2 ug estradiol/female/day for 14 days, or 4 mg estradiol/female/day for 14 days. Administration of psoralen increased the tyrosine content of both white and black skins, with higher concentrations in white skin than black. Hydrocortisone increased the tyrosine content in white skin, but did not affect black skin. Testosterone propionate and estradiol both decreased tyrosine in white and black skin. This decrease may be due to increased incorporation of the amino acid into protein. (16 Refs)

51. MULTIPLE FORMS OF HUMAN TUMOR CALCITONIN DEMONSTRATED BY DENATURING POLYACRYLAMIDE GEL ELECTROPHORESIS AND LECTIN AFFINITY CHROMATOGRAPHY.

Baylin SB, Wieman KC, O'Neil JA, Roos BA

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J Clin Endocrinol Metab; 53(3):489-497 1981

The mol wt forms of immunoreactive calcitonin in human tumors have been studied using two sodium dodecyl sulfate polyacrylamide gel electrophoretic systems. Under denaturing conditions, which produce monomer peptides, it has been possible to document further the size heterogeneity of this immunoreactivity. Small mol wt forms (native size human calcitonin of 3,600 daltons and immunoreactive peptides of 4,000-6,000 daltons) predominated in extracts of four medullary thyroid carcinomas. Smaller amounts of larger mol wt forms (8,000-22,000 daltons) were detected. In one medullary thyroid carcinoma and in an extract of small (oat) cell lung carcinoma, the higher mol wt forms predominated, with the greatest amount of immunoreactivity found in 12,000-and 10,000-dalton peptides. Evidence has been obtained that all of the tumors studied contain a component of immunoreactive calcitonin that binds to lectins immobilized on agarose. Five to 20% of the total immunoreactive material from the medullary thyroid carcinomas and from human small (oat) cell lung carcinoma bound to columns of agarose-bound Concanavalin A and/or wheat germ agglutinin. The conditions of binding and elution with specific sugars strongly suggest that this lectin-bound immunoreactive calcitonin may contain mannose and N-acetylglucosamine residues. Gel electrophoresis of the carbohydrate-eluted material showed that the lectin-bound forms comprise a portion of each of the several mol wt classes of immunoreactive calcitonin moieties identified. These studies indicate further that human tumors contain different sizes of calcitonin immunoreactivity and that the ratios among these forms may vary among different neoplasms. The fact that some calcitonin forms may contain carbohydrate moieties is another potential determinant for the heterogeneity of the circulating immunoreactive calcitonin in patients with medullary thyroid car cinoma or small (oat) cell lung cancer. (Author abstract) (40 Refs)

52. LEUCINE METABOLISM IN PATIENTS WITH MALIGNANT DISEASE.

Waterhouse C, Mason J

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Cancer; 48(4):939-944 1981

Studies of the metabolism of the amino acid, leucine, have been carried out in six patients with progressive cancer and compared to similar studies in a group of control subjects. U-14C leucine was given by the single injection technique and the disappearance of radioactive leucine from the plasma as well as the appearance of the label in expired air was determined over a 3-hr interval in both the overnight fasting state and during glucose ingestion. Plasma leucine levels in the basal state were higher in the patients with malignant disease (193 +-17 umol/L vs 135 +-16 umol/L) as was the flux or rate of appearance of unlabeled leucine molecules into the plasma (136 +-5 umol/kg hr vs 79 umol/kg/hr). Oxidation of leucine resulted in the appearance of about 19% of the injected label in expired CO2 of control subjects with almost twice that amount being found in progressive cancer patients. When the irreversible disposal of leucine to a metabolic fate other than oxidation was calculated (perhaps an indicator of protein synthesis), this was also increased in the progressive cancer patients. Exogenous glucose administration resulted in a fall in both leucine level and flux in the control subjects, while these parameters were less affected in three of the cancer patients. The fall in oxidation when glucose was given was more pronounced in the control subjects. These data are interpreted to mean that all aspects of leucine metabolism, presumably an example of branched chain amino acid metabolism, appear to be accelerated in subjects with cancer and wt loss. In addition, the peripheral control of the metabolism of this group of amino acids, as affected by glucose and insulin, appears to be impaired. (Author abstract) (16 Refs)

53. SURPRISING DIFFERENCES IN SUBSTRATE SELECTIVITY AND OTHER PROPERTIES OF SYSTEMS A AND ASC BETWEEN RAT HEPATOCYTES AND THE HEPATOMA CELL LINE HTC.

Handlogten ME, Garcia-Canero R, Lancaster KT, Christensen HN

(c/o Christensen) Dept. Biological Chemistry, Univ. Michigan, Ann Arbor, MI, 48109

J Biol Chem; 256(15):7905-7909 1981

We find that the two wide-range Na+-dependent transport systems A and ASC for various neutral amino acids can be discriminated more sharply in the hepatoma cell line HTC than in any cell yet studied by us in which the two systems co-exist. The gain comes partly from a higher reproducibility and a higher relative ASC rate for HTC than in ordinary rat hepatocytes, also a repressed condition of System A unless first deprived of amino acids, but mainly from our finding that in the hepatoma cell threonine serves as a nearly specific substrate and inhibitor of System ASC, thus decisively supplementing older discriminatory techniques. In ordinary hepatocytes cysteine is quite specific to ASC as a substrate but not as an inhibitor, whereas threonine is specific in neither role. In the hepatoma cell cysteine in turn is specific in neither role. In addition to these and other differences between the two cells in analog specificity, which are partly assignable to System ASC and partly to System A, System ASC of the hepatoma cell shows an inhibition on lowering the pH from 6.5 to 5 not seen in the ordinary hepatocyte. Furthermore, threonine uptake by the hepatoma cell undergoes no stimulation when Li+ is substituted for choline in a Na+-free medium, whereas ASC uptake by the ordinary rat hepatocyte is stimulated much as is System A uptake. As in other occurrences, and in contrast to System A, ASC transport in the hepatoma cell is stimulated neither by amino acid deprivation nor by insulin, glucagon, or dexamethasone. Trans-stimulation, both inward and outward, via System ASC is vigorous in the hepatoma cell. Despite the surprising differences observed, common features of each system in various occurrences continue to justify the use of the abbreviations ASC and A as long as they are understood as generic designations. (Author abstract) (15 Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Adnet J-J 10
Alcaraz G 11
Aoyagi T 43
Bakker GH 41
Banerjee PK 50
Banks J 12
Baylin SB 51
Bersch N 18
Bhavanandan VP 12
Bigotti A 20
Birembaut P 10
Borenfreund E 7
Borner JW 24
Bounous G 49
Bourgois A 11
Bradshaw TK 42
Brennan MF 37
Briggs RC 2
Brouty-Boye D 40
Burns GF 26
Buzby GP 45
Carubelli R 31
Cavaliere R 20
Chen LB 40
Cheng YS 40
Christensen HN 53
Condie RM 24
CrossCE 38
Davidson EA 12
De Rossi G 25
Denk H 7
Dickey WD 15
Dousset H 10
Dressel T 24
Engelhardt DL 44
Feldmann RJ 21
Fellini SA 22
Ferrone S 20
Ferrone S 28
Fishman WH 36
Frangione B 17
Franke WW 7
Franklin E 17
Freedman RB 42
Frick MP 24
Fryd DS 24
Furmanski P 47
Gaillard D 10
Gajl-Peczalska 24
Garcia-Canero R 53
Geiger B 4
Gerdes J 29
Gerriets JE 38
Glass WF 2
Glaudemans CP 21
Goding JW 26
Golde DW 18
Goodale RL 24
Groopman JE 18
Handlogten ME 53
Hascall VC 22
Hascall VC 23
Havsteen B 27
Havsteen B 29
Hebbeln J 27
Hemmingsen L 16
Hesterberg TW 38
Hnilica LS 2
Hoffman-Liebermm 39
Hoogerbrugge JW 41
Husby G 19
Iacobelli S 25
Iizuka H 43
Imai K 20
Imai K 28
Ishigami H 13
Jackson AC 38
Jones GR 46
Jordan B 11
Kaderbhai MA 42
Kalis B 10
Kamigaki K 43
Katlick AW 12
Kawakami H 34
Kemper JG 12
Kimura JH 22
Kimura JH 23
Kishore GS 31
Kitta D 46
Klatt U 29
Klatzow DJ 14
Klebe RJ 15
Kongshavn PA 49
Kootstra A 1
Kostka G 9
Kube D 47
Kunz W 46
Labat-Robert J 10
Lancaster KT 53
Laskin JD 44
Last JA 38
Leutenegger M 10
Liebermann D 39
Likovsky Z 48
Longo P 25
MacLeod MC 1
Mandelli F 25
Mansfield BK 1
Mason J 52
Mastrangelo R 25
Mayer D 7
Meza AT 33
Michaelsen T 19
Miura Y 43
Mondal M 50
Motoi S 5
Mufson RA 44
Mullen JL 45
Muller V 29
Nakajima T 5
Nakajima T 6
Nakajo S 32
Nakamura Y 32
Nakaya K 32
Namba M 35
Natali PG 20
Natali PG 28
Nathan I 18
Natoli V 25
Natvig JB 19
Norton JA 37
Nose K 35
O'Leary J 24
O'Neil JA 51
Okamoto H 35
Oshima RG 3
Osswald H 46
Pang KC 49
Park C 47
Pasqualetti D 25
Pellegrino MA 20
Piccinini L 44
Poole AR 23
Postel E 27
Potter M 21
Poynard J-P 10
Quan SG 18
Ranelletti FO 25
Rasmussen F 16
Recht B 17
Reiner A 23
Reiser KM 38
Rieber M 30
Rieber M 33
Rieber MS 30
Robert L 10
Rommerts FF 41
Roos BA 51
Rosato EF 45
Roth H 46
Sachs L 39
Sadarangani C 49
Sandoval IV 8
Saruta M 43
Sato Y 5
Sato Y 6
Schlessinger J 4
Schmid E 7
Schmidt WN 2
Schweiger A 9
Seals CM 15
Selkirk JK 1
Sire J 11
Skaarup P 16
Slaga TJ 1
Sletten K 19
Smetana K 48
Stein H 29
Stein TP 37
Stein TP 45
Stryjecka-Zimme 2
Taylor T 24
Terayama H 34
Thoenes J 29
Thonar EJ 23
Tokumitsu K 36
Tokumitsu S 36
Van Logheim E 17

Van der Molen H. 41
Vandekerckhove 8
Vos GH 14
Warthold U. 27
Watanabe S. 5
Watanabe S. 6
Waterhouse C. 52
Weinstein IB. 44
Westerbrink K. 27
Westermark P. 19
Wieman KC. 51
Wilson BS. 20
Wilson BS. 28
Wolf H. 16
Yang NS. 47

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In this issue:

- **NUCLEOPROTEINS** **1-2**
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** **3-11**
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** **12-26**
- **BINDING/ RECEPTOR PROTEINS** **27-29**
- **MEMBRANE/ SURFACE PROTEINS** **30-34**
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** **35**
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** **36-39**
- **DIETARY AND NUTRITIONAL EFFECTS** **40**
- **OTHER RELATED STUDIES** **41-46**

NUCLEOPROTEINS

1. HUMAN LIVER NUCLEOLAR ANTIGENS.

Busch RK, Busch H
Dept. Pharmacology, Baylor Coll. Medicine, Houston, TX, 77030
Proc Soc Exp Biol Med; 168(1):125-130 1981

Normal human liver nucleoli were used to immunize rabbits and the resultant antibodies were used to study antigens in human liver and other tissues. Immunofluorescence studies with the antibodies to human liver nucleoli, showed nucleoli as bright fluorescent organelles against a less fluorescent nuclear background. In most tissue sections, a limited number of fluorescent nucleoli were observed. Nuclear membrane fluorescence was frequently observed. In HeLa cells, the localization

HE2. 3173/2.

of fluorescence was less restricted. Nucleolonemal and nuclear network fluorescence was visible. In the placental sections, few nucleoli were found and more background fluorescence was noted. Immunodiffusion analysis revealed two immunoprecipitin bands which appeared to be liver specific. Other immunoprecipitin bands were common to liver, placenta, and HeLa nuclear extracts. Rocket immunoelectrophoresis revealed two liver-specific antigens (L1 and L4), one migrating to the cathode and the other to the anode. Thus liver nucleolar-specific antigens, not found in Hela placental cells, were identified. It was suggested that since a neoplasm may result from a loss of normal protein, information on normal human liver nucleolar and nuclear antigens may aid in the detection of specific proteins lost in neoplasia. (16 Refs)

2. HISTONE VARIANT COMPOSITION OF NORMAL AND LEUKAEMIC HUMAN LYMPHOCYTES: ANALYSIS BY GEL ELECTROPHORESIS OF WHOLE CELLS AND NUCLEI.

Froussard P, Tempelis LD, Levy SB
(c/o Levy) Div. Hematology-Oncology, Tufts-New England Medical Center, 136 Harrison Ave., Boston, MA, 02111
Br J Haematol; 49(2):275-282 1981

The histone variant composition of normal and leukemic lymphocytes was analyzed using a method which circumvented endogenous cellular proteolysis. Whole cells were solubilized in buffer containing protamine which released the histones from the DNA and allowed their immediate analysis by Triton X-100-acetic acid - urea gel electrophoresis. The results were comparable to those obtained with histones which had been acid-extracted from cells, nuclei or chromatin; however, the new method was more reproducible since proteolysis was controlled. By histone variant analysis, chronic lymphocytic leukemia patients could be separated into two groups. One group had lymphocytes with a histone H2a variant ratio of about 1.0; this finding resembled lymphocytes (75-85% T cells) from normal individuals. The larger group had lymphocytes with a reproducibly lower histone H2a variant ratio; these cells had a relative increase in the second variant, H2a.2. The two groups of patients could not be distinguished by clinical disease state. No other differences were noted in the histone variant composition of lymphocytes from normal and leukemic individuals. (Author abstract) (17 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

3. A NEW SMALL (40 KD) KERATIN FILAMENT PROTEIN MADE BY SOME CULTURED HUMAN SQUAMOUS CELL CARCINOMAS.

Wu YJ, Rheinwald JG
Div. Cell Growth and Regulation, Sidney Farber Cancer Inst., 44 Binney St., Boston, MA, 02115
Cell; 25(3):627-635 1981

We examined the keratin proteins of nine cell lines cultured from human squamous cell carcinomas (SCCs) of the epidermis and oral epithelium. All of these lines contained the four major keratins (46 kd, 50 kd, 56 kd, and 58 kd) found in normal keratinocytes cultured from these epithelia. A 40 kd protein was also present in the Triton-insoluble cytoskeletal fraction of three of the lines. The 40 kd protein had an isoelectric point of 5.3, near that of the 46 kd and 50 kd keratins. It was precipitated by an antiserum against total s corneum keratins, and also by an antiserum against purified 46 kd keratin. An antiserum raised against the purified 40 kd protein had a higher titer against the 40 kd protein, detected by immunoprecipitation, and did not crossreact with the 46-58 kd keratins. The anti-40 kd serum stained, by indirect immunofluorescence, a cytoplasmic filament network in cells of the SCC lines that synthesize the 40 kd protein. These filaments were of a pattern indistinguishable from that stained by antiserum against total s corneum keratins, and the pattern was not altered by pretreatment of the cells with colcemid. The anti-40 kd serum did not stain the SCC lines that do not synthesize the 40 kd protein; normal human newborn foreskin keratinocytes or fibroblasts; or 3T3, HeLa or XB cells. Keratinocytes cultured from uninvolved epidermal or oral tissue of patients whose tumors had given rise to SCC lines that synthesize the 40 kd protein were also not stained by the anti-40 kd serum. Synthesis of the 40 kd protein was greatly reduced in tumors formed by the SCC lines that make this protein in culture. Partial proteolytic digestion maps of the 40 kd protein were unique, but very similar to those of the 46-58 kd keratins. These results constitute strong evidence that the 40 kd protein is a keratin, and is neither a breakdown product nor an incomplete translation product of a gene for one of the larger keratins. It is the smallest epidermal keratin yet to be described. The 40 kd keratin may be the product of a normally unexpressed gene or transcript that often becomes activated during the course of keratinocyte transformation. Like the larger keratins, the level of its expression is regulated and is different in cells growing in vivo and in culture. (Author abstract) (37 Refs)

4. TUMOR AND PLACENTAL HISTAMINASE. I. AFFINITY CHROMATOGRAPHY PURIFICATION AND CHARACTERIZATION OF THE PLACENTAL ENZYME.

Lin CW, Kirley SD, StPierre M
Urology Res. Lab., Massachusetts General Hosp., Boston, MA, 02114
Oncodev Biol Med; 2(4):267-280 1981

An improved affinity-chromatography purification procedure for human placental histaminase (an enzyme also associated with a number of cancers) that yields a homogeneous product is reported together with the molecular characterization of the purified enzyme. A cadaverine-AH-sepharose gel was prepared as the affinity medium; the improvement in the procedure consists in eluting the gel with chromotropic acid, which released 85% of the enzyme from the column at 100 milliM, compared to only 18% released with the use of heparin. The results of both gel electrophoresis and immunodiffusion assays for purity showed that the histaminase prepared by the modified technique is homogeneous. Polyacrylamide gel electrophoresis studies indicated that the minimum subunit mol wt of histaminase is approx 90,000 daltons (range 91,000-110,000). Amino acid composition determinations on histaminase hydrolyzed for 24, 48, and 72 hr revealed a relatively low content of sulfur-containing amino acids and a high content of dicarboxylic amino acids. Isoelectric focusing studies in polyacrylamide gel of both the crude placental enzyme extract and the purified enzyme gave identical patterns, with at least five major and several diffuse bands, indicating the structure of the enzyme was not changed by the purification process. (29 Refs)

5. PLATELET ADENINE NUCLEOTIDES AND ARACHIDONIC ACID METABOLISM IN THE MYELOPROLIFERATIVE DISORDERS.

Russell NH, Salmon J, Keenan JP, Bellingham AJ
Dept. Hematology, Royal Liverpool Hosp., Liverpool, England
Throm Res; 22(4):389-397 1981

Experiments were carried out in 17 patients with various myeloproliferative disorders (prior to any therapy) to determine the relationship between abnormalities of platelet aggregation to platelet adenine nucleotide pools, platelet cyclo-oxygenase (CO) and lipoxygenase (LO) activity, and thromboxane B2 (TxB2) synthesis. Ten patients had abnormalities of platelet aggregation to ADP and/or collagen; 5/10 had bleeding tendencies; all had significantly diminished total and releasable adenine nucleotide pools compared with 7 other patients and controls. In all patients, CO activity as measured by malondialdehyde production in response to exogenous sodium arachidonate was normal; CO from 5 patients with abnormal aggregation followed Michaelis-Menten kinetics. Two of the patients with abnormal platelet aggregation has diminished LO activity as measured by the synthesis of 12-hydroxy-5,8,10,14-eicosatetraenoic acid. There was no statistical difference between TxB2 synthesis in patients with abnormal platelet aggregation and controls when measured by a radioimmunoassay. The results of the CO, LO, and TxB2 studies show that prostaglandin synthesis is normal in these patients and confirms the importance of an acquired storage pool deficiency in the etiology of the platelet defect and clinical bleeding in myeloproliferative diseases. (28 Refs)

6. IMMUNOCHEMICAL IDENTIFICATION OF INTERMEDIATE-SIZED FILAMENTS IN HUMAN NEOPLASTIC CELLS. A DIAGNOSTIC AID FOR THE SURGICAL PATHOLOGIST.

Gabbiani G, Kapanci Y, Barazzone P, Franke WW
Dept. Pathology, Univ. Geneva, 40 Boulevard de la Cluse, 1211 Geneva 4, Switzerland
Am J Pathol; 104(3):206-216 1981

Forty-three tumors were investigated by means of immunofluorescence with the use of antibodies against the following different classes of intermediate-sized (10 nm) filament proteins: (1) cytokeratins, (2) vimentin, and (3) desmin. In general, the immunologic features of tumor-cell intermediate filaments are those present in their tissue of origin. It can be seen, therefore, that, during neoplastic transformation, there are no major changes in the synthesis of the type of intermediate filament proteins when compared to normal tissues. Immunologic identification of these proteins furnishes the surgical pathologist with a quick and clear-cut way to differentiate tumors of mesenchymal origin from epithelial neoplasms, and in particular to distinguish between malignant lymphomas and lymph node metastases of undifferentiated carcinomas. (Author abstract) (43 Refs)

7. IN VIVO STUDIES OF ACTIN-CONTAINING CYTOSKELETAL ABNORMALITIES IN NEOPLASTIC CELLS.

McNutt NS
Veterans Admin. Medical Center, 4150 Clement St., San Francisco, CA, 94121
Eur J Cell Biol; 25(1):218-222 1981

The role of cytoskeletal abnormalities in the spread of malignant cells and metastasis is discussed. The possibility that defective control of cell motility is expressed in actin-containing cytoskeletal alterations accompanying malignant tumor invasion is considered. Projections into the basement membrane of carcinomas arising from stratified squamous epithelium have been observed. Wide-necked pseudopods 3 um or more in diameter and projecting into the basement membrane are likely to be significant for tumor invasion. The formation of pseudopodia needs to be accompanied by sufficient changes in cell-to-cell adhesive contacts to allow cells to translocate into the basement membrane. Cell junctional contacts observed in adenocarcinomas and sarcomas are described. The possible involvement of host response in bringing about the cytoskeletal abnormalities and the motile behavior of malignant cells is considered. (57 Refs)

8. IMMUNOHISTOCHEMICAL STUDIES OF THE LOCALIZATION OF PROTEIN S-100 AND GLIAFIBRILACIDIC PROTEIN (GFAP) IN HUMAN BRAIN TUMOR TISSUE (MEETING ABSTRACT).

Ishizeki J, Ishida Y
First Dept. Pathology, Sch. Medicine, Gunma Univ., Maebashi-shi, Gunma Prefecture 371, Japan
Program and Abstracts of the 22nd Workshop on Selected Topics of the Japanese Society of Neuropathology held in Fukuoka, Japan, 9-11 May, 1981. The Japanese Society of Neuropathology, Kyoto 602, Japan, 136 pp., 1981.

By using the antiserum for GFAP and protein S-100, obtained from a human brain tumor and bovine brain, respectively, 13 astrocytomas, 14 glioblastomas, 8 oligodendrogliomas, 3 ependymomas, 1 subependymoma, 1 choroid plexus papilloma, 5 medulloblastomas, 10 meningiomas, 4 neurinomas, 2 angioblastomas, 3 pituitary adenomas, 1 craniopharyngioma and 1 metastatic carcinoma were examined for GFAP and S-100. The astroglial nucleus and cytoplasm constituting the astrocytoma contained protein S-100 and the cytoplasmic projections were stained with the anti-GFAP serum. The Rosenthal fibers were GFAP positive, and the corpora amylacea were protein S-100 positive. The response of the cells of malignant astrocytoma and glioblastoma varied from cell to cell reflecting cell atypicality. Most of the cells in the oligodendroglioma which shows a clear halo had neither protein. However, these tumors often contained many GFAP- and protein S-100-positive astrocytes, showing a mixed constitution. The cytoplasmic projections of the ependymoma, which show a perivascular rosette, were stained with protein S-100 and GFAP. Glial fibers containing both proteins were the basic structure of the subependymoma. Most of the medulloblastomas examined contained neither protein, but some of the tumors contained cells which had both proteins. GFAP- and S-100 protein-positive cells were found in the pale area of the desmoplastic medulloblastoma and the angioblastomas. The cells constituting the neurinomas and the choroid plexus papilloma contained protein S-100. Meningioma, pituitary adenoma, metastatic carcinoma and craniopharyngioma contained neither protein. (no Refs) (In JPN)

9. CHARACTERIZATION OF ARGINASE ACTIVITY FROM MOUSE EPIDERMIS AND ITS RELATION TO ORNITHINE DECARBOXYLASE INDUCTION BY THE TUMOR-PROMOTING AGENT, 12-O-TETRADECANOYLPHORBOL-13-ACETATE.

Verma AK, Boutwell RK
Dept. Human Oncology, Wisconsin Clinical Cancer Center, Univ. Wisconsin Center for Health Sciences, Madison, WI, 53792
Biochim Biophys Acta; 677(2):184-189 1981

Arginase, which catalyzes the cleavage of L-arginine to urea and ornithine, was detected in both soluble and particulate fractions of mouse epidermis. In a typical experiment, about 75 and 25% of the total arginase activity was associated with the soluble (100,000 x g supernatant) and the washed particulate fraction, respectively. Both soluble and particulate enzymes required the presence of divalent Mn++ for activity. Arginase activity was increased by about 50% in the particulate fraction, but not in the soluble fraction, by preheating the fractions at either 50 or 55 C in the presence of 15 mM $MnCl_2$. Enzyme activity in both fractions, in the absence of 15 mM $MnCl_2$ dropped precipitously during heating. A comparison of the nature of arginases in the soluble and particulate fractions revealed similar Km values (13 mM) and pH optima (9.5) and identical heat denaturation curves. Application of 10 nmol of 12-O-tetradecanoylphorbol-13-acetate to mouse skin did not increase arginase activity in either fraction over a period of 24 hr. In contrast, there was a large increase in ornithine decarboxylase activity in the soluble fraction 4.5 hr after treatment. Mouse epidermal ornithine decarboxylase activity was much less than arginase activity and was predominantly localized in the soluble fraction. These results indicate that the normal level of arginase activity is not a limiting factor for the stimulation of polyamine biosynthesis by TPA. High arginase activity in mouse epidermis may play a role in providing ornithine for polyamine biosynthesis and in the production of glutamate and proline as well as in the production of keratinous proteins. (Author abstract) (23 Refs)

10. DEOXYATP-RESISTANT RIBONUCLEOTIDE REDUCTASE OF MUTANT MOUSE LYMPHOMA CELLS. EVIDENCE FOR HETEROZYGOSITY FOR THE PROTEIN M1 SUBUNITS.

Eriksson S, Gudas LJ, Ullman B, Clift SM, Martin DW
Univ. California, San Francisco, CA, 94143
J Biol Chem; 256(19):10184-10188 1981

A somatic mutant cell line of murine T-lymphoma cells (S49), dGuo-200-1, has been isolated and contains a ribonucleotide reductase with altered allosteric properties. The enzyme in dGuo-200-1 extracts was inhibited only 50% by the allosteric inhibitor, dATP. Thus, half of the ribonucleotide reductase activity appeared totally resistant to dATP inhibition, suggesting that the mutant cells possess two types of enzyme, one sensitive and one resistant. We have purified the ribonucleotide reductase from mutant and wild type cells in order to test this hypothesis and gain insights into the regulatory properties of this key enzyme in deoxyribonucleotide biosynthesis. Ribonucleotide reductase consists of two nonidentical subunits, proteins M1 and M2. They can be completely separated by chromatography on dextran blue-Sepharose to which protein M1 binds, but protein M2 does not. When proteins M1 and M2 were separated from dGuo-200-1 extracts, it could be demonstrated by reconstitution experiments that the dATP resistance was a property of protein M1. The protein M1 subunit was purified by dATP-Sepharose chromatography to near-homogeneity. The activity from the mutant cells chromatographed as two peaks. One eluted at 3 to 6 mM ATP and was insensitive to dATP inhibition, while the other eluted at 10 to 20 mM ATP and was sensitive to dATP inhibition. The latter peak cochromatographed with wild type protein M1. Thus, dGuo-200-1 cells contain two protein M1 components, a presumed mutant allele product and a wild type allele product, both present in approx equal amounts in extracts from the heterozygous cells. (Author abstract) (26 Refs)

11. ACTIN DEPOLYMERIZING ACTIVITY IN TUMOUR-BEARING RATS.

Goodlad GA, Clark CM
Dept. Biochemistry and Microbiology, Univ. St. Andrews, Irvine Building, Fife, KY16 9AL, Scotland
Cancer Lett; 14(1):1-5 1981

The actin-depolymerizing activity present in the plasma, liver and gastrocnemius muscle of normal and tumor-bearing Wistar rats and in the Walker tumor grown in solid and ascitic form was measured. A 3-fold increase in the level of unpolymerized actin in platelet-free plasma from the tumor-bearing rat was observed, while no alteration in plasma actin-depolymerizing activity was observed. In the liver, the level of actin-depolymerizing activity was unaffected by tumor growth. The level of unpolymerized actin in gastrocnemius muscle was very low in comparison to liver, and increased in the tumor-bearing animal. Actin-depolymerizing activity in gastrocnemius muscle was very low compared to liver, but was unaffected by tumor growth. The actin-depolymerizing activity per mg in the two tumor forms did not differ significantly. Apparently, there is a rise in the level of unpolymerized actin in liver, plasma and gastrocnemius of rats bearing a transplantable Walker 256 carcinoma and the level of unpolymerized actin in the cells of this tumor growing sc is considerably higher than when it is grown in the ascitic form. However, no accompanying alteration in actin-depolymerizing activity was observed in any of the tissues studied. The changes in the state of polymerization of actin in the tissues of tumor-bearing rats could not be attributed to an effect of the tumor on actin depolymerizing activity. (12 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

12. A MONOCLONAL IMMUNOGLOBULIN G1 IN WHICH SOME MOLECULES POSSESS GLYCOSYLATED LIGHT CHAINS - I. SITE OF GLYCOSYLATION.

Savvidou G, Klein M, Horne C, Hofmann T, Dorrington KJ
Dept. Biochemistry and Pathology, Univ. Toronto, Toronto M5S 1A8, Canada
Mol Immunol; 18(9):793-805 1981

A human monoclonal IgG1kappa protein (Hom) was found to possess two species of light chain with mol wt of 23,000 and 28,000 respectively. The difference in mass was due to the presence of a carbohydrate prosthetic group on the 28,000 dalton species. The two species of light chain were separated by chromatography on CM-cellulose. Circular dichroism failed to detect any significant differences in conformation between the glycosylated and non-glycosylated forms and both species recombined with a heavy chain in an identical fashion as judged by difference spectroscopy. An anti-idiotypic antibody raised against the glycosylated Fab-fragment of IgG1Hom was unable to distinguish between this species and the non-glycosylated Fab fragment, suggesting that the structure of the combining site is identical or very similar in both molecules. Amino-acid sequence analyses showed that both light chains had an identical sequence up to residue 41. Further studies on the glycosylated light chain, using o-iodosobenzoic acid fragments, localized the carbohydrate attachment site to asparagine 107, the penultimate residue of the Jkappa region which links Vkappa to Ckappa. The sequence around the attachment site was Asn-Arg-Thr which satisfies the requirements for an acceptor sequence. The non-glycosylated light chain was found to have the same sequence in this region indicating that the absence of glycosylation was not due to the lack of an acceptor sequence. A kinetic mechanism has been proposed to account for the incomplete glycosylation of the light chains of IgG1Hom in which there is a competition between the rate of folding of the nascent polypeptide and the rate of attachment of core sugars via the dolichol pyrophosphate pathway. (Author abstract) (40 Refs)

13. SYNTHESIS OF PRO-COLLAGEN TYPE II BY A XENOTRANSPLANTED HUMAN CHONDROBLASTIC OSTEOSARCOMA.

Lanzer WL, Liotta LA, Yee C, Azar HA, Costa J
(c/o Liotta), NIH, Building 10, Room 8B19, Bethesda, MD, 20205
Am J Pathol; 104(3):217-226 1981

The chondroblastic component of a human osteosarcoma was established as a transplantable tumor in nude athymic mice for the first time. The collagen biosynthetic production of the excised tumor was studied in organ culture. The majority of the collagen secreted in the culture media was pro-Type II as identified by diethylaminoethyl cellulose (DEAE cellulose) chromatography, gel electrophoresis, and cyanogen bromide cleavage. This system should be useful for producing large quantities of human pro-Type II collagen and for studying neoplastic cell switching of collagen synthesis. (Author abstract) (17 Refs)

14. EFFECT OF PLASMINOGEN ACTIVATOR (UROKINASE), PLASMIN, AND THROMBIN ON GLYCOPROTEIN AND COLLAGENOUS COMPONENTS OF BASEMENT MEMBRANE.

Liotta LA, Goldfarb RH, Brundage R, Siegel GP, Terranova V, Garbisa S
Lab. Pathophysiology, NCI, Bethesda, MD, 20205
Cancer Res; 41(11,part1):4629-4636 1981

This study evaluated the role of the serine proteases urokinase (plasminogen activator), plasmin, and another regulatory protease, alpha-thrombin, in the degradation of the basement membrane (BM). Homogeneously pure enzyme preparations were incubated with isolated components of BM and with whole human amnion BM. Urokinase failed to significantly degrade fibronectin or any of the other BM components. Under 25 C (native) conditions, plasmin and thrombin cleaved fibronectin and laminin into multiple specific fragments but did not produce a major cleavage of collagenous BM components. Alpha-Thrombin selectively degraded only the mol wt 400,000 chain of laminin, whereas plasmin degraded both of the laminin chains. Digestion of laminin by the serine proteases was time and concentration dependent. Type V collagen was cleaved by thrombin and plasmin at 35 C but not at temperatures below 33 C. Immunohistology studies indicated that laminin, but not Type IV collagen, was removed from the whole BM by plasmin treatment. The results suggest that the BM components are poor substrates for plasminogen activators and that plasmin alone is not sufficient to completely degrade the whole BM. Plasmin, generated through the action of plasminogen activator, may play a significant role in the degradation of noncollagenous components of the BM. (51 Refs)

15. THE CSF-PROTEIN PATTERN IN ACUTE CEREBELLAR ATAXIA OF CHILDHOOD AND INTRACRANIAL MIDLINE TUMOURS.

Siemes H, Siegert M, Jaroffke B, Hanefeld F
Arbeitsgruppe 'Klinische und Experimentelle Plasmaproteinforschung', Kinderklinik der Freien Universitat, Heubnerweg 6, D-1000 Berlin, W. Germany
Eur J Pediatr; 137(1):49-57 1981

The protein pattern in childhood acute cerebellar ataxia (ACA) was analyzed to elucidate its pathogenesis. The protein profile in intracranial midline tumors (IMT) was characterized. The patterns of both diseases were compared to obtain information useful for their differential diagnosis. Lumbar cerebrospinal fluid (CSF) from 251 patients (3 mo to 15 yr old; 25 ACA, 39 IMT, 61 aseptic meningitis, 40 bacterial meningitis, and 86 with no CNS disease) was examined. Of the 25 ACA children, 10 showed slightly elevated CSF cell counts. Significant changes in the eight CSF-protein fractions were observed in all ACA children. The CSF cell count was elevated in 10/14 children with medulloblastomas, 2/9 with pontine gliomas, and in none of the remaining tumor patients. Tumor cells were identified in six children with medulloblastomas and in none of the other children. Total protein was frequently increased in association with medulloblastomas (12/14 cases) and rarely in the other tumor patients (4/25). Significant changes of the protein pattern in children with brain tumor were found in nearly all the fractions, giving a pattern similar to that in acute nonbacterial meningitis. The children with medulloblastomas showed a marked increase of gamma3, which comprises most of the IgG. Oligoclonal gamma-globulin amounts to 1.9-12.4% of total protein. Differentiating ACA and IMT by CSF-protein analysis is possible. The mechanisms of CSF changes in children with ACA and IMT are discussed. (23 Refs)

16. PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN LEUKOCYTE INTERFERON (IFLRA) WITH MONOCLONAL ANTIBODIES.

Staehelin T, Hobbs DS, Kung H, Lai CY, Pestka S
Pharma Res. Div., F. Hoffmann-La Roche and Company, Ltd., CH-4002, Basel, Switzerland
J Biol Chem; 256(18):9750-9754 1981

Recombinant human WBC interferon produced in bacteria (IFLrA) was purified to homogeneity with the use of monoclonal antibodies against WBC interferon. The purified interferon exhibited a single band of Mr = approx 19,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Amino acid analysis and the NH2-terminal sequence were consistent with the sequence predicted from the DNA. Some of the purified product contained NH2-terminal methionine; the terminal methionine was removed from the rest of the chains. (Author abstract) (18 Refs)

17. ACUTE PHASE REACTANTS AND CLINICAL STAGES IN MULTIPLE MYELOMA.

San Miguel J, Vicente V, Battle J, Hernandez F, Borrasca AL
Dept. Hematology, Univ. Hosp., Salamanca, Spain
Neoplasma; 28(3):333-338 1981

Serum levels of seven specific proteins mostly acute phase reactants (APR) have been studied (transferrin, alpha2-macroglobulin, alpha1-antitrypsin, haptoglobin, C3, ceruloplasmin, orosomucoid) in 14 healthy subjects and in 55 patients with multiple myeloma. The alpha2-macroglobulin and transferrin are significantly decreased in the myeloma group compared with healthy controls whereas the remaining proteins under study are elevated, significantly only orosomucoid and ceruloplasmin. The APR in the IgG3 myelomas seem to show differences from those of the IgG1 and IgG2 subclasses the levels of the negatively changed proteins being lower, whereas the positive APR higher in the IgG3 myeloma. In the IgA myeloma, however, decreased levels of haptoglobin and alpha1-antitrypsin have been found in spite of being positive APR. Transferrin and haptoglobin serum level can be included as a new parameter in regression equations for calculation of IgA myeloma cell mass. (Author abstract) (21 Refs)

18. PANCREATIC POLYPEPTIDE. A REVIEW.

Lonovics J, Devitt P, Watson LC, Rayford PL, Thompson JC
(c/o Thompson) Dept. Surgery, Univ. Texas Medical Branch, Galveston, TX, 77550
Arch Surg; 116(10):1256-1264 1981

Pancreatic polypeptide (PP), a 36-amino acid peptide, may function as an important feedback inhibitor of pancreatic secretion after a meal. It arises from both islet and acinar cells of the pancreas. Release of PP by a meal, primarily protein, occurs in a biphasic manner. The first rapid release occurs as a result of vagal stimulation; the second, more prolonged rise (the so-called intestinal phase) occurs in response to hormonal stimulation, predominantly cholecystokinin. Plasma PP levels increase with age; PP levels are elevated above those of age-controlled normal subjects in diabetic patients and in some patients with pancreatic amine precursor uptake decarboxylase tumors. The value of plasma PP as a possible marker for pancreatic tumors is as yet unsettled but may be a valuable tool. (Author abstract) (136 Refs)

19. OROSOMUCOID, ALPHA2-MACROGLOBULIN AND IMMUNOGLOBULINS IN SERUM AND PLEURAL EFFUSIONS.

Asseo PP, Tracopoulos GD
(c/o Tracopoulos), 10th Medical Unit Athens Chest Hosp. 'Sotiria', Messogeion 152, Athens, Greece
Am J Clin Pathol; 76(4):437-441 1981

Serum and pleural fluid levels of orosomucoid, alpha2-macroglobulin, IgG, IgA, and IgM were measured in 36, 29, and 9 patients suffering from malignant, tuberculous, and non-malignant, non-inflammatory pleural disease, respectively. Pleural fluid concentrations of the studied proteins were higher in tuberculous effusions, but due to wide overlapping of the values, correlation with diagnosis could not be established. The ratio of the pleural fluid to the serum concentration of the studied proteins was below unity with the exception of three tuberculous and one cancer patient who showed higher values of orosomucoid in the pleural fluid. The rate of diffusion of the various proteins in the pleural fluid was inversely proportional to their mol wt. (Author abstract) (8 Refs)

20. IMMUNOGLOBULINIC FRAGMENTS OF URINARY PROTEINS IN PATIENTS AFFECTED BY ACUTE PNEUMOPATHIES, LUNG CANCER, AND TUBERCULOSIS.

Scrobogna S, Vriz G, Torre R, Trani S, Novello E, Panfili E
Laboratorio di Analisi, Ospedale Specializzato Regionale Pneumologico 'Santorio Santorio', via Bonomea 265, 34136 Trieste, Italy
Quad Sclavo Diagn; 17(1):31-39 1981

Electrophoretic and immunoelectrophoretic tests were performed on 95 urine samples of patients with lung cancer (15), bronchopneumonia (15), active tuberculosis (10), decompensated cardiopathy (10), chronic nephropathy (5), and chronic bronchitis (40). Of the 15 lung cancer patients 11 had the highest gamma globulin concentrations. Gamma fraction represents 20-40% of total proteins. Immunoelectrophoresis revealed a pattern: absence or only traces of alpha and mu anti-gamma, but marked increase in anti-lambda and kappa precipitation [F(ab) and Fc]. Proteins migrating in the gamma position are mostly fragments with mol wt below albumins. An increase in immunoglobulin synthesis and catabolism is apparent. End to inflammatory processes was preceded by disappearance of abnormal proteins; reduction in urinary immunoglobulinic fragments occurred after surgical removal of neoplasia and after immunosuppressive therapy. (21 Refs) (In ITA)

21. MALIGNANT TUMORS OF THE HEAD AND NECK AND BIOCHEMICAL MARKERS.

Doi Y, Takeuchi T, Hotate T, Kataura A, Nishio M, Sakurai T, Kamata N, Uchida M
Dept. Otolaryngology and Oral Surgery, Tonan Hosp., Sapporo, Japan
Gan No Rinsho; 27(11):1309-1317 1981

Concentrations of beta-aminoisobutyric acid (bAIBA) and beta-alanine (bA) were measured in the urine of 117 patients with malignant tumors of the head and neck and were compared to those in 158 patients with benign diseases of the head and neck who were thought to have high urinary concentrations of bAIBA and bA. Overall, 82/117 patients with malignant tumors had high concentrations of urinary bAIBA and 19/117 had high concentrations of urinary bA; 87/117 had high concentrations of either bAIBA or bA. Overall, 15/158 patients with benign diseases had high concentrations of bAIBA and 6/158 had high concentrations of bA; 18/158 had high concentrations of either bAIBA or bA. Histologically, high concentrations of bAIBA were observed in 66/82 patients with squamous cell carcinoma, and either bAIBA or bA concentrations were high in 68/82 patients with squamous cell carcinoma and 8/10 with malignant lymphoma. Compared to patients with squamous cell carcinoma, a higher percentage of the patients with malignant lymphoma had high concentrations of bA. bAIBA and bA were thought to have applications in the diagnosis of malignant tumors. (23 Refs) (In JPN)

22. NEW CLASS OF TRANSFORMING GROWTH FACTORS POTENTIATED BY EPIDERMAL GROWTH FACTOR: ISOLATION FROM NON-NEOPLASTIC TISSUES.

Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB
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Proc Natl Acad Sci USA; 78(9,part2):5339-5343 1981

Proteins potentiated by epidermal growth factor (EGF) to induce a transformed phenotype in non-neoplastic rat kidney fibroblasts in cell culture have been isolated from many non-neoplastic tissues of the adult mouse, including submaxillary gland, kidney, liver, muscle, heart, and brain. They resemble previously described transforming growth factors (TGFs) isolated from neoplastic cells as follows: they are extractable by acid/ethanol and are acid-stable, low mol wt (6,000-10,000) polypeptides requiring disulfide bonds for activity, and they cause anchorage-independent growth of non-neoplastic indicator cells that will not grow in soft agar in their absence. Partial purification of these TGFs from submaxillary glands of male mice shows that they are distinct from EGF. Unlike previously described extracellular TGFs, but like certain cellular TGFs from neoplastic cells, they are potentiated by EGF in their ability to promote anchorage-independent growth. The isoelectric point of the submaxillary gland TGF protein is near neutrality. Chromatography on Bio-Gel P-30 followed by high-pressure liquid chromatography has resulted in a 22,000-fold overall purification. The most purified protein is active in inducing growth in soft agar in 1 ng/ml when assayed in the presence of EGF. The data add further evidence to the concept that neoplasia may result from a quantitative, rather than qualitative, alteration in non-neoplastic biochemical processes. (Author abstract) (25 Refs)

23. CHARACTERIZATION OF IMMUNOLOGICALLY SIGNIFICANT UNIQUE B16 MELANOMA PROTEINS PRODUCED IN VIVO AND IN VITRO.

Gersten DM, Hearing VJ, Marchalonis JJ
Dept. Pathology, Georgetown Univ. Medical Center, Washington, DC, 20007
Proc Natl Acad Sci USA; 78(8):5109-5112 1981

Proteins unique to B16 melanoma cells grown in culture and as solid tumors have been independently described recently. As both proteins have apparent Mr of 65,000-70,000, we have more fully compared their biochemical characteristics. The Mr 65,000 protein, which is isolated from B16 cells in culture, has an aggregate mol wt of greater than 200,000 under nonreducing conditions. This Mr 65,000 form is shed into the culture medium exclusively and is the predominant molecular species of serum-free culture supernatants. It was found that B700, a major membrane protein of B16 cells in solid tumors, and the Mr 65,000 preparation from cultured cells were electrophoretically identical when compared under four separate sets of conditions. These two proteins also share immunologic determinants. The results suggest the identity of these two proteins isolated by completely different approaches. (Author abstract) (22 Refs)

24. EPIDERMAL CELL (KERATINOCYTE)-DERIVED THYMOCYTE-ACTIVATING FACTOR (ETAF).

Luger TA, Stadler BM, Katz SI, Oppenheim JJ
NIH, Natl. Inst. Dental Res., Bldg. 30, Room 324, Bethesda, MD, 20205
J Immunol; 127(4):1493-1498 1981

In order to determine whether keratinocytes play a role in the modulation of the immune response, we investigated the murine keratinocyte cell line Pam 212. In culture these cells generate a substance with a biologic activity that greatly enhances phytohemagglutinin-induced thymocyte proliferation. We have, therefore, called this substance epidermal cell thymocyte-activating factor (ETAF). This keratinocyte-derived supernatant activity is mainly produced at the onset of the logarithmic growth phase and is directly mitogenic for murine thymocytes. Although ETAF by itself exhibits no T cell growth factor activity, ETAF enhances interleukin 2 production by mitogen-stimulated murine spleen cells. Murine ETAF is not genetically restricted and lacks species specificity since it decreases lectin-induced proliferation of human peripheral blood lymphocytes (as well as murine spleen cells) and also enhances the production of human interleukin 2. The factor has a mol wt between 15,000 and 25,000 as determined by gel filtration and elutes as a single peak from anion exchange chromatography columns. The activity is maintained mainly at alkaline pH and is rapidly destroyed at temperatures above 60 C. These observations suggest that epidermal cells may interact with the immune system by elaborating nonspecific factors that modulate lymphocyte proliferation and augment lymphokine production. (Author abstract) (41 Refs)

25. RAT MAMMARY CARCINOMA CELLS SECRETE ACTIVE COLLAGENASE AND ACTIVATE LATENT ENZYME IN THE STROMA VIA PLASMINOGEN ACTIVATOR.

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Inst. Dental Res., United Dental Hosp. Sydney, Chalmers St., Surry Hills 2010, Australia
Int J Cancer; 28(4):509-515 1981

A spontaneous mammary adenocarcinoma (AC) from an inbred female rat was investigated with regard to secretion of neutral proteases. Cultures of neoplastic epithelial cells derived from the tumor secreted an enzyme that fulfilled the criteria for a specific collagenase. In contrast to cultures of non-neoplastic cells, tumor collagenase was present as an active enzyme, since treatment with trypsin or p-aminophenylmercuric acetate (APMA) did not increase activity. The neoplastic cells were also prolific producers of plasminogen activator (PA). Dexamethasone (Dex)(10(-6) M) markedly reduced the levels of both enzymes. Addition of tranexamic acid (TA), an inhibitor of plasmin and of plasminogen activation, did not affect collagenase activity, even at 10(-1) M TA, nor did latent collagenase accumulate. Latent collagenase was secreted in culture by normal fibroblasts from neonatal rat lungs. This latent enzyme was activated by the addition of tumor cell medium plus plasminogen, but this effect was inhibited by the addition of TA. These results demonstrate that the neoplastic cells themselves secrete collagenase as an active enzyme. PA is also secreted, is not involved with tumor collagenase, but is capable, in the presence of plasminogen, of activating latent collagenase produced by the non-neoplastic cells within the tumor or in the surrounding tissue. This tumor possesses potent collagenolytic ability in vitro which may be partly responsible for its rapid invasion in vivo. (Author abstract) (36 Refs)

26. BIOCHEMICAL PROPERTIES OF GLOMERULAR BASEMENT MEMBRANE IN DAUNOMYCIN NEPHROSIS AND NEPHROTOXIC SERUM NEPHRITIS.

Koide H, Soeda N, Ohno J
Div. Nephrology, Dept. Medicine, Juntendo Univ. Sch. Medicine, Tokyo, Japan
Renal Physiol; 4(2/3):102-107 1981

The chemical compositions of glomerular basement membranes (GBM) from normal rats and rats with nephrosis induced by daunomycin (DN), and nephrotoxic serum nephritis (NSN) were compared. Glomerulonephritis was induced in rats by injection of 1.2 mg daunomycin/100g body wt iv. Rats were killed after 3 wk when urinary protein excretion was greater than 400 mg/24 hr. NSN was induced by injection of rabbit antiserum against rat GBM (0.5 or 0.8 ml/100g body wt). Analysis of amino acid composition in DN rats showed a decrease in the amount of 4-hydroxyproline, glycine, methionine, and hydroxylysine, and an increase in the relative amounts of serine, glutamic acid, leucine, and arginine. The ratios of 3-hydroxyproline to 4-hydroxyproline (3HP/4HP) and 4-hydroxyproline to proline (4HP/P) were unchanged. GBM from NSN rats showed decreased 4-hydroxyproline, glycine, isoleucine, and half-cystine, and increased aspartic acid, threonine, serine, glutamic acid, alanine, leucine, tyrosine, hydroxylysine, and lysine. The 3HP/4HP ratio was the same as in controls, while the 4HP/P ratio was significantly reduced. Carbohydrate analysis showed an increase in glucosamine in DN GBM and decreased fucose content and increased sialic acid content in NSN GBM. NSN GBM also showed reduced glucosyl-galactosyl-hydroxylysine. Results suggest a decrease in the collagenous component and an increase in the non-collagenous component of GBM in DN and NSN. (8 Refs)

BINDING/ RECEPTOR PROTEINS

27. CHARACTERIZATION OF THE ASIALOGLYCOPROTEIN RECEPTOR IN A CONTINUOUS HEPATOMA LIN E.

Schwartz AL, Fridovich SE, Knowles BB, Lodish HF
Biology Dept., Massachusetts Inst. Technology, Cambridge, MA, 02139
J Biol Chem; 256(17):8878-8881 1981

The asialoglycoprotein receptor has been identified on a continuous human hepatoma cell line, HepG2. This receptor requires Ca++ for ligand binding and is specific for asialoglycoprotein. There are approx 150,000 ligand molecules bound/cell at 4 C. These receptors represent a homogeneous population of high affinity binding sites with Kd = 7 x 10(-9) M. From the rate of 125I-ASOR binding at 4 C, kon was 0.95 x 10(6) M-1 min-1. Uptake of 125I-ASOR at 37 C was approx 0.02 pmol/min/10(6) cells. (Author abstract) (22 Refs)

28. ESTROGEN AND PROGESTERONE BINDING PROTEINS IN HUMAN COLORECTAL CANCER. A PRELIMINARY CHARACTERIZATION OF ESTRADIOL RECEPTOR.

Sica V, Contieri E, Nola E, Bova R, Papaleo G, Puca GA
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Tumori; 67(4):307-314 1981

Estradiol receptor (ER) and progesterone receptor (PgR) were assayed in tumors from 20 patients with primary colorectal cancer. Ten of 20 tumors contained high affinity sites for 17beta-estradiol and progesterone. The highest concentration of ER was 56 fmol/mg of protein. The ER dissociation constant ranged from 1.6 x 10(-10) M to 8 x 10(-10) M (mean 4.6 +-2.6). The highest concentration of PgR was 42 fmol/mg of protein. The PgR dissociation constant ranged from 3 x 10(-9) to 9 x 10(-9) M (mean 5.65 +-2.1). Four out of 20 specimens analyzed were from male patients and all resulted negative for both receptors. Sixty percent of ER positive tumors were also PgR positive, whereas only 20% of ER negative were PgR positive. Sucrose gradient centrifugation showed that cytoplasmic ER of colorectal cancer sedimented at 3 S in the absence of protease inhibitors and at 4.5 S in the presence of 1 mM phenylmethylsulphonyl fluoride (PMSF) both in low and in high ionic strength. When chromatographed on Sephadex G-200 almost all ER was quantitatively recovered in the included fractions. Mol wt of ER eluted from Sephadex G-200 ranged from 90,000 to 50,000 daltons. Elution profile and mol wt heterogeneity suggest that, in spite of the presence of PMSF, there is a limited proteolysis of ER. Partially purified colorectal cancer ER did not bind to sepharose-heparin. The isoelectric point of ER was 6.4-6.5. (Author abstract) (35 Refs)

29. GALACTOSYL SPECIFIC RECEPTOR ON LIVER CELLS: BINDING SITE FOR TUMOR CELLS.

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Eur J Cell Biol; 25(1):95-102 1981

In this paper the recognition of various rat tumor cells by rat liver cells is d emonstrated in vitro. A liver cell receptor involved in the binding process has been identified. The ultrastructure of the cell contacts was examined by transmission electron microscopy. Hepatocytes and Kupffer cells were isolated from rat liver by collagenase treatment and cell adhesion tests were performed with four different tumor cell types. Hepatocytes were found to bind Walker sarcoma cells, lymphoma cells and Yoshida hepatoma cells but not leukemia 5222 cells. Kupffer cells bound all tumor cell types. Normal blood cells were not bound under the same conditions. Recognition of tumor cells by hepatocytes was mediated by a galactose specific lectin on the liver cell surface as shown by hapten inhibition experiments with specific saccharides. Although Kupffer cells express a similar lectin-like receptor adhesion of tumor cells, they could not or could only slightly be inhibited by galactose or related saccharides. It is concluded that the spontaneous adhesion of tumor cells to liver cells in vitro is a specific recognition event which in part is mediated by lectin-carbohydrate interactions. (Author abstract) (33 Refs)

MEMBRANE/ SURFACE PROTEINS

30. ISOLATION AND PARTIAL CHARACTERIZATION OF A MUCIN-TYPE GLYCOPROTEIN FROM PLASMA MEMBRANES OF HUMAN MELANOMA CELLS.

Umemoto J, Bhavanandan VP, Davidson EA
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Biochim Biophys Acta; 646(3):402-410 1981

Plasma membranes were isolated from HM7 melanoma cells grown in the presence of [3H]glucosamine and Na35/2SO4 or [3H]mannose and [14C]glucosamine. The labeled glycoconjugates were solubilized with 0.6 M lithium diiodosalicylate/0.5% Triton X-100. Fractionation of glycoconjugates by repeated chromatography on columns of Sepharose CL-6B and DEAE-Sepharose and by affinity chromatography on WGA-Sepharose yielded three radiochemically homogenous glycoproteins. One of these having an apparent mol wt of 100,000 was found to contain clusters of (AcNeu)1 or 2--[Gal--GalNAc] linked O-glycosidically to the protein. One other glycoprotein contained both O-glycosidically and N-glycosidically-linked oligosaccharides, and the third contained only N-glycosidically-linked carbohydrates. Preliminary results indicate that the 100,000 mol wt mucin-type glycoprotein is present in significantly reduced quantities in cultured human fetal uveal melanocytes. Further, the bulk of the glycoproteins from the melanocytes were of lower molecular size compared to those from the melanoma cells. (Author abstract) (33 Refs)

31. CELL ADHESION-DEPENDENT DIFFERENCES IN ENDOGENOUS PROTEIN PHOSPHORYLATION ON THE SURFACE OF VARIOUS CELL LINES.

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Biochim Biophys Acta; 670(2):274-284 1981

Endogenous phosphorylation of intact cells was studied with four mouse, hamster and human cell lines using [gamma-32P]ATP and [gamma-32P]GTP as exogenous substrates. With all four cell lines distinct differences in the phosphoprotein patterns could be demonstrated for cells grown in suspension culture compared to cells grown in monolayers. Two major, apparently ubiquitous phosphoproteins with mol wt of 135,000 (128,000 in HeLa cells) and 105,000, representing up to 60% of total phosphorylation, were phosphorylated only in cells grown in suspension. These phosphoproteins and the kinase(s) were located on the surface of the suspension cells. Evidence showed that phosphorylation was apparently not a true endogenous reaction, that rather it occurred by cell-cell collision, showing exponentially increasing 32P incorporation with increasing cell population density, Phosphorylation of pp135 and pp105 was established with ATP as well as with GTP and was not dependent on cyclic nucleotides cyclic AMP, cyclic GMP and cyclic CMP. The substrate-attached cells of all four cell lines have protein kinases on the cell surface. The lack of pp135 and pp105 phosphorylation may be due to the fact that these phosphoproteins are not expressed at all on the surface of substrate-attached cells or that these phosphoproteins are already fully phosphorylated. (Author abstract) (32 Refs)

32. TRANSFORMATION-DEPENDENT QUANTITATIVE CHANGES IN GLYCOPEPTIDE BINDING TO CONCANAVALIN A-SEPHAROSE.

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Cancer Lett; 14(1):55-62 1981

Quantitative changes in concanavalin A (Con A)-Sepharose binding of differentially L-fucose-labeled glycopeptides from a newly established Syrian golden hamster (SGH) fetal lung cell line as a control, and transformed and tumor cell lines derived from the SGH line, were investigated. Cochromatography on Sephadex G50 was used to detect transformation-dependent mol wt changes in the glycopeptides of the new cell lines. M3E3/C3 is the established cell line from SGH fetal lung, SA 17, SA Ru, and SA F1 were chemically transformed clones, SA17T, SAF1T, and SA RuT were established by recultivation of dissected solid tumors induced in allogeneic hosts after injection of transformed lines. Con A-Sepharose chromatography demonstrated that for SA 17T and M3E3/C3 lines, surface glycopeptides were composed of predominantly non-binding material, while the main part of control glycopeptides was bound to the column. In the tumor lines, the part of non-binding glycopeptides was further augmented and the binding part was concomitantly decreased compared to the transformed parent line. In all transformed and tumor cell lines investigated, t he quantitative change in Con A binding was paralleled by a shift of transformed or tumor glycopeptides to higher apparent mol wt compared to the control in gel chromatography. (24 Refs)

33. MOLECULAR PATHOLOGY OF CANCER CELL ADHESIVENESS.

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Dept. Pathology, Kumamoto Univ. Medical Sch., 2-2-1 Honjo-machi, Kumamoto 860, Japan

Acta Pathol Jpn; 31(4):623-640 1981

A new cell surface-associated adhesive glycoprotein with a mol wt of 70,000 was separated from differentiated rat ascites hepatoma cells forming cell islands in vivo (but not from undifferentiated rat ascites hepatoma cells present as single cells in vivo) and highly purified by chromatography; it was synthesized by the cells and localized on the cell surface. Its synthesis began to rise rapidly and reached its peak in 24 hr cultivation, ie, a 10-fold increase. This substance induced not only aggregation but also adhesiveness of the cells characterized by junctional complexes including tight junctions, desmosomes, and intermediate junctions, closely resembling the frequency and distribution of junctional complexes observed on the above cells islands. Its potency was inhibited specifically by D-mannose and alpha-methyl-D-mannoside; the numbers of the binding sites per cell were calculated as 6 x 10(5). Its activity was concerned with the protein portion of the molecule, and not with the carbohydrate portion. Thus, it seemed reasonable that the adhesive glycoprotein may play a key role in the cell adhesiveness and island formation. In contrast, serum-associated adhesive glycoprotein, separated from normal rat serum, could aggregate the cells but not develop junctional complexes. (Author abstract) (45 Refs)

34. EPINEPHRINE DESENSITIZATION OF ADENYLATE CYCLASE FROM CYC- AND S49 CULTURED LYMPHOMA CELLS.

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J Cyclic Nucleotide Res; 7(3):161-172 1981

Specific beta-adrenergic desensitization of adenylate cyclase (AC) was characterized in the AC deficient lymphoma cell line cyc- and in the wild type S49 (WT). Cells were preincubated with (-)epinephrine (EN)plus 1.0 milliM thiourea and 0.1 milliM ascorbate or with thiourea and ascorbate alone. Both cyc- and WT cells were desensitized at the same rate by 1.0 uM EN during the first 10 min after treatment. After 60 min WT cells were 75% desensitized and cyc- cells 54% desensitized. Pretreatment for 18 hr with the beta-adrenergic agonist terbutaline produced EN-stimulated desensitization of 92% in WT compared with 48% in cyc-. Beta-adrenergic receptor levels were reduced in WT from 321 to 28 femtomoles (fmol)/mg protein and in cyc- from 580 to 438 fmol/mg protein. Both WT and cyc- were resensitized by propranol ol, although WT did not recover full sensitization. Half max desensitization was produced in both cell types by 0.2-0.4 uM EN, and full desensitization by 10 uM EN. Apparently both WT and cyc- undergo rapid, reversible specific beta-adrenergic desensitization of AC that is independent of the G/F proteins of the adenylate cyclase complex. A slower desensitization occurs only in WT. (27 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

35. POLYAMINE CONCENTRATION, ACTIVITY OF THE RIBOSOMAL DISSOCIATION FACTOR AND PROPORTION OF RIBOSOMES ACTIVE IN PROTEIN SYNTHESIS IN THE 4-DIMETHYLAMINOAZOBENZENE-INDUCED RAT HEPATOMA.

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Cancer Lett; 14(1):77-83 1981

The activity of the ribosomal dissociation factor in the high salt ribosomal wash and in the particle free cytoplasm of 4-dimethylaminoazobenzene (DAB)-induced liver tumors as compared to the livers of control rats was investigated and the proportion of ribosomes active in protein synthesis and the concentration of the polyamines spermine, spermidine and putrescine were determined. While the spontaneous dissociation of ribosomal preparations from DAB hepatoma was higher than from control liver, the addition of ribosomal wash extract and supernatant extract from the hepatoma caused a decrease in dissociation. The addition of control liver dissociation factor extracts to normal ribosomal preparations caused an increase in dissociation into active subunits. The proportion of ribosomes active in protein synthesis as indicated by sucrose density gradient analysis was significantly lower in tumor tissue. A significant decrease of putrescine in the DAB tumor cell extracts was noted, compared to normal rat liver. The spermidine/putrescine and (spermidine plus spermine)/putrescine ratios increased, predisposing towards association of the subunits. (22 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

36. BIOSYNTHESIS AND HORMONE-REGULATED EXPRESSION OF SECRETORY GLYCOPROTEINS IN RAT LIVER AND HEPATOMA CELLS. EFFECT OF GLUCOCORTICOIDS AND INFLAMMATION.

Baumann H, Held WA
Dept. Cell and Tumor Biology, Roswell Park Memorial Inst., Buffalo, NY, 14263
J Biol Chem; 256(19):10145-10155 1981

Exposure of rat hepatoma tissue culture cells to dexamethasone results in appearance of a new glycoprotein, gp35-50 (Mr = 35,000 to 50,000) and increased synthesis of another glycoprotein, gp50 (Mr = 50,000). The glycoproteins synthesized in a fractionated cell-free translation system (containing dog pancreas microsomes) appear as a well separated series of spots on two-dimensional polyacrylamide gels which differ in size and charge. Glycoproteins synthesized by glucose-starved cells show similar size and charge heterogeneity. The size heterogeneity consists of a series of spots, each differing in mol wt by about 3,000, which could be almost completely abolished by treatment with endo-beta-N-acetylglucosaminidase H. Our results indicate that unglycosylated gp50 (Mr = 42,000) typically acquires three N-glycan units, whereas gp35-50 (Mr = 22,000) possesses eight N-glycosylation sites. Analysis of the cell-free translation products directed by mRNA from hepatoma tissue culture cells grown in tissue culture, from hepatoma tissue, and from normal liver tissue indicated that administration of dexamethasone causes a pronounced increase in gp35-50 mRNA in all three tissues. A similar increase was observed in liver after inflammation which along with other biochemical properties suggests that gp35-50 may be alpha1-acid protein. In contrast, mRNA coding for gp50 was not increased by dexamethasone in tumor tissue and no protein structurally related to gp50 was detected in the liver mRNA translation products. Thus, gp35-50 is expressed in normal liver, whereas gp50 is expressed in hepatoma cells and is differentially regulated by steroid hormones depending on whether the cells are grown in tissue culture or as a tumor in the rat. (Author abstract) (53 Refs)

37. TUMOR-PROMOTING PHORBOL DIESTER-INDUCED SPECIFIC CHANGES IN CELL SURFACE GLYCOPROTEIN PROFILE OF K562 HUMAN LEUKEMIC CELLS.

Fukuda M
Biochemical Oncology, Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
Cancer Res; 41(11,part1):4621-4628 1981

The tumor-promoting phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), induced specific changes in the cell surface glycoprotein profile of K562 human leukemic cells. TPA-treated K562 cells were examined by cell surface labeling followed by immunoprecipitation with anti-glycophorin antiserum or anti-K562 antiserum. TPA-treated K562 cells lost glycophorin and glycoprotein with a mol wt of 105,000, which are specific to later stages of erythroid cell differentiation, and retained glycoprotein with a mol wt of 95,000, which is specific to early stages of erythroid cell differentiation. On the other hand, 4alpha-phorbol-12,13-didecanoate, which is inactive as a tumor promoter, induced no significant changes in cell surface glycoproteins of K562 cells. In parallel, TPA induced morphological changes of K562 cells as their growth was inhibited, whereas 4alpha-phorbol-12,13-didecanoate had no effect on these two characteristics. Thus, the tumor promoter induced specific changes in the cell surface glycoprotein profile of K562 human leukemic cells, which appeared to be induced into a retrodifferentiated stage. (Author abstract) (51 Refs)

38. TAXOL INDUCES THE ASSEMBLY OF FREE MICROTUBULES IN LIVING CELLS AND BLOCKS THE ORGANIZING CAPACITY OF THE CENTROSOMES AND KINETOCHORES.

De Brabander M, Geuens G, Nuydens R, Willebrords R, De Mey J
Lab. Oncology, Janssen Pharmaceutica, B-2340 Beerse, Belgium
Proc Natl Acad Sci USA; 78(9,part2):5608-5612 1981

Taxol, a potent promoter of microtubule polymerization in vitro, induces massive assembly of free microtubules in cultured cells as visualized by immunocytochemistry and electron microscopy. The centrosomes and kinetochores largely lost their capacity to organize microtubule assembly, as became evident by the disappearance of the cytoplasmic microtubule complex and the mitotic spindle. The taxol-induced microtubules were partially resistant to nocodazole, an inhibitor of tubulin polymerization. Moreover, taxol induced microtubule assembly in cells pretreated with nocodazole. Increasing the ratio of nocodazole to taxol restored the ability of the centrosomes and kinetochores to specifically induce microtubule assembly in their immediate vicinity. The data suggest that taxol lowers the critical tubulin concentration in vivo as well as in vitro and that the organizing capacity of the microtubule-organizing centers depends on the cytoplasmic polymerization threshold. (Author abstract) (20 Refs)

39. AFFINITY LABELING OF CHICKEN LIVER DIHYDROFOLATE REDUCTASE BY A SUBSTITUTED 4,6-DIAMINODIHYDROTRIAZINE BEARING A TERMINAL SULFONYL FLUORIDE.

Kumar AA, Mangum JH, Blankenship DT, Freisheim JH
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J Biol Chem; 256(17):8970-8976 1981

Chicken liver dihydrofolate reductase is rapidly and stoichiometrically inactivated by a substituted 4,6-diaminodihydrotriazine containing a terminal benzenesulfonylfluoride (DTBSF). The substrate dihydrofolate largely prevents the enzyme inhibition by DTBSF, whereas NADPH had no effect, indicating that the inhibitor is bound at or near the folate site. Using radiolabeled inhibitor between 1.0 and 1.2 mol was incorporated/mol of enzyme (Mr = 21,651), following treatment with 8 M urea at 75 C. Digestion of the maleylated, radiolabeled inhibitor-enzyme complex with trypsin and subsequent gel filtration on Sephadex G-50 SF yielded a single major peak of radioactivity. The covalently modified limited tryptic peptide was subsequently purified to homogeneity using high performance liquid chromatography. The radiolabeled tryptic peptide had the following sequence: Asn-Glu-Tyr (DTBS)-Lys-Tyr-Phe-Gln-Arg (residues 29-36). Automated Edman degradation of this peptide revealed that the radioactivity derived from the inhibitor was released at Step 3, identifying tyrosine-31 as the specific site of covalent attachment of the affinity label. (Author abstract) (24 Refs)

DIETARY AND NUTRITIONAL EFFECTS

40. BODY COMPOSITION AND DIETARY INTAKE IN NEOPLASTIC DISEASE.

Cohn SH, Gartenhaus W, Vartsky D, Sawitsky A, Zanzi I, Vaswani A, Yasumura S, Ra i K, Cortes E, Ellis KJ
Medical Res. Center, Brookhaven Natl. Lab., Upton, NY, 11973
Am J Clin Nutr; 34(10):1997-2004 1981

Changes in body composition in 37 cancer patients were studied over a period of 6 mo. Total body nitrogen was measured by prompt gamma neutron activation; total body potassium by whole body counting, and total body water with the use of the tritium isotope. The nitrogen balance of patients with neoplastic diseases varied from marked negative to positive. Initially, the patients were divided into two groups: those who lost body wt (over 10%) and those who maintained or gained body wt before the study. Analysis of body composition indicated that patients who lost body wt had caloric and protein intakes markedly below 'normal' levels at the beginning of the study. There also appears to be a direct relationship between the protein intake and the total body potassium/total body water ratio in the cancer patients. At the end of the 6-mo study, the patients were again placed into two groups on the basis of wt loss or gain (and maintenance). Changes in body composition over the period were analyzed in terms of lean body mass, its protein constituent, water, and fat. Wt loss was found to reflect primarily the loss of fat, water, lean body mass (potassium), and only to a minor extent the protein component of lean body mass (nitrogen). Further, on the basis of the values of the ratios of total body nitrogen/total body potassium/total body water, it was possible to ascertain the relative normalcy of the body tissue gained or lost in the 6-mo period. The results of the present study suggest that the ratio total body nitrogen/total body potassium may serve as the best indicator of recent or ongoing catabolism or anabolism of the neoplastic process. By means of the application of the techniques used in this study for the determination of body composition, it should be possible to assess regimes of hyperalimentation for cancer patients who lose body wt. (Author abstract) (13 Refs)

OTHER RELATED STUDIES

41. EFFECT OF EXOGENOUS METHIONINE DEPLETION ON PROTEIN SYNTHESIS IN NORMAL AND LEUKEMIC LEUKOCYTES CULTURED IN VITRO.

Tautt J, Maj S, Chlopkiewicz B, Koziorowska J
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Acta Haematol Pol; 12(2):69-75 1981

The effect on protein synthesis of methionine deprivation or of its replacement by homocysteine in the culture medium was studied in peripheral blood lymphocytes and leukocytes from 8 healthy donors and 17 adult patients with acute leukemia (10 with acute myeloblastic leukemia and 7 with acute lymphoblastic leukemia). The effect was evaluated by labeling experiments with tritiated valine. Protein synthesis by lymphocytes and leukocytes of healthy donors was significantly inhibited in the absence of methi onine, but there was no significant difference in inhibition between methionine-free cultures and those cultures in which methionine was replaced by homocysteine. Similar findings were obtained for the leukemic leukocytes as well in the 34-hr culture. Here, the protein synthesis was inhibited by 0% in 4/17 cases, and by 15-50% in 13/17 in the absence of methionine; by 0% in the same 4 cases, and by 18-57% in the presence of homocysteine. (12 Refs) (In POL)

42. THE EFFECT OF LOW-MOLECULAR-WEIGHT INHIBITORS ON THE GROWTH IN VITRO OF A HUMAN PANCREATIC ADENOCARCINOMA CELL LINE.

Grant AG, Grant DA
Dept. Surgery, St. George's Hosp. Medical Sch., Cranmer Terrace, London SW17 0RE, England
Biochem Soc Trans; 9(1):134 1981

Inhibition of growth in an in vitro human pancreatic exocrine adenocarcinoma cell line was studied. Low mol wt proteinase inhibitors included antipain and leupeptin (active against trypsin-like enzymes), pepstatin (active against pepsin-like enzymes), and elastatinal (active against elastase-like enzymes). Growth was decreased 50% by 5-25 uM leupeptin or antipain. Cell numbers were reduced by 50 uM leupeptin to 35% and by 50 uM antipain to 25% of control values. Pepstatin and elastinal had no effect. [3H]-thymidine labeling occurred in 63% of controls within 24 hr. Doubling time was delayed 24 hr by 5 uM leupeptin. Antipain (5 uM) had no effect on cell division rate, but decreased the number of cells reaching interphase. Reseeding of cells in medium without inhibitors resulted in return to normal growth rate, suggesting that the inhibition was reversible. No cell toxicity was noted for 5-25 uM antipain or leupeptin. In tests to quantify the amount of inhibitor in culture medium by measuring inhibition of trypsin activity in cell-free medium, trypsin hydrolysis was reduced to a constant value regardless of whether the medium was assayed before, during, or after growth studies. This indicated that the inhibitors were not taken up by cells, but acted extracellularly. Results suggest that in vivo use of proteinase inhibitors for treatment of tumors would require long-term administration. (6 Refs)

43. EVIDENCE FOR GENETICALLY INDEPENDENT ALLOSTERIC REGULATORY DOMAINS OF THE PROTEIN M1 SUBUNIT OF MOUSE RIBONUCLEOTIDE REDUCTASE.

Eriksson S, Gudas LJ, Clift SM, Caras IW, Ullman B, Martin DW
Univ. California, San Francisco, CA, 94143
J Biol Chem; 256(19):10193-10197 1981

Ribonucleotide reductase is responsible for the reduction of the 2'-hydroxy moiety of all four ribonucleoside diphosphates to the corresponding deoxyribonucleotides. The overall activity of the enzyme is regulated by the allosteric effectors ATP (activator) and dATP (inhibitor), and the enzyme's substrate specificity is also controlled by nucleotide effectors. For instance, wild type ribonucleotide reductase from mouse T-lymphoma (S49) cells require dGTP as a positive effector for ADP reduction. This effect of dGTP causes a reciprocal inhibition of CDP reduction. The dGuo-L mutant cell line, resistant to growth inhibition by exogenous deoxyguanosine, contains a nucleotide-binding subunit, protein M1, that conveys to its CDP reductase an insensitivity to dGTP (and dTTP) inhibition. The dGuo-L protein M1 also shows a decreased capacity to use ADP as a substrate, and therefore, the regulation of the substrate specificity is altered in the mutant protein M1. Another mutant cell line, dGuo-200-1, is resistant to deoxyadenosine and its ribonucleotide reductase is abnormally resistant to inhibition by dATP. The isolated mutant protein M1 from dGuo-200-1 cells has a CDP reductase activity which is stimulated by dATP, unlike the wild type enzyme which is inhibited by dATP. It appears that this mutant enzyme has lost the capacity to distinguish between dATP and ATP, but is still sensitive to regulation by dGTP and dTTP. Thus, the site of protein M1 regulating overall activity is altered in the dGuo-200-1 mutant, while the site regulating substrate specificity is normal. These characteristics of the mutants provide genetic evidence for two independent allosteric domains of protein M1, each responsible for a different aspect of nucleotide sensitivity of ribonucleotide reductase. (Author abstract) (21 Refs)

44. INTERACTION OF 2-AMINOBICYCLO[3.2.1]OCTANE-2-CARBOXYLIC ACID WITH THE AMINO ACID TRANSPORT SYSTEMS OF THE SARCOMA 37 MURINE ASCITES TUMOR CELL.

Elliott MS, Matthews RH, Minton JP, Zand R
(c/o Matthews) Dept. Physiological Chemistry, Ohio State Univ., Columbus, OH, 43210
Biochemistry; 20(18):5105-5108 1981

The relatively broad and overlapping specificities of amino acid transport systems have made the synthesis of analogues specific to single transport systems desirable. The analogue in general use as a specific substrate for transport system L has been 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH). The affinity of BCH for the binding site of system L has been shown to be less than that of the natural substrate, leucine. Earlier studies from this laboratory suggested that higher homologues in a series could have greater affinity for system L. A higher homologue of BCH, 2-aminobicyclo[3.2.1]octane-2-carboxylic acid (ABOCA), has been synthesized and studied as a substrate and competitor for amino acid transport systems of the sarcoma 37 (S37) ascites cell. ABOCA inhibited the transport system dominant in the low concentration region for histidine uptake (system L) but had no effect on the uptake of labeled N-methyl-alpha-aminoisobutyric acid (MeAIB). MeAIB had no effect on labeled ABOCA uptake in S37 cells. ABOCA inhibited the uptakes of labeled leucine and labeled BCH competitively. Leucine, histidine, and BCH inhibited the uptake of labeled ABOCA competitively. Typical L system substrates demonstrated exchange effects with labeled ABOCA. The b isomer of ABOCA demonstrated slightly greater affinity for system L than did the a isomer. We conclude that ABOCA is an analogue restricted to interaction with amino acid transport system L, that it has greater affinity for system L than does BCH, and that its selection for system L is determined principally by an apolar interaction with steric considerations secondary. (Author abstract) (18 Refs)

45. EFFECT OF THE TUMOR PROMOTER 12-O-TETRADECANOYLPHORBOL-13-ACETATE ON ALPHA-AMINOISOBUTYRATE UPTAKE IN FRIEND ERYTHROLEUKEMIC CELLS.

Becker JH, Cook JS
(c/o Cook) Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
Cancer Res; 41(11,part1):4512-4517 1981

In Friend erythroleukemic cells, the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) increases the rate of influx as well as the steady-state accumulation of the amino acid transport probe alpha-aminoisobutyric acid (AIB). This non-metabolizable analog is concentrated in these cells by utilizing the energy of the Na+ electrochemical gradient. The effect of TPA is to increase the ouabain-sensitive component of uptake of AIB (and of the K+ analog 86Rb+). Transport changes are not observed until 30 min after application of the promoter and reach a max in about 6 hr. The effects are blocked by cycloheximide. The flux results are consistent with a model in which the membrane potential is increased and, with it, the driving force on the coupled Na+ -AIB movement. The effect is possibly mediated by a change in electrogenicity of the Na:K pump. TPA raises the steady-state accumulation levels of AIB. By altering external K+ in the presence of valinomycin, the membrane potential may be adjusted and, with it, the accumulation of AIB. Over a wide range of membrane potentials, TPA-treated cells accumulate more AIB than do controls, suggesting a possible change in the internal Na+ activity. (Author abstract) (35 Refs)

46. A COMPARATIVE STUDY OF THE DISTRIBUTION OF THE STABLE CROSSLINK, PYRIDINOLINE, IN BONE COLLAGENS FROM NORMAL, OSTEOBLASTOMA, AND VITAMIN D-DEFICIENT CHICKS.

Yamauchi M, Banes AJ, Kuboki Y, Mechanic GL
Dental Res. Center 210-H, Univ. North Carolina, Chapel Hill, NC, 27514
Biochem Biophys Res Commun; 102(1):59-65 1981

To evaluate the mechanism of bone collagen maturation, distribution of pyridinoline (PD) was studied in tryptic peptides from bone collagen of normal, avian osteosarcoma (A0)-bearing, and vitamin D-deficient chicks. Gel filtration chromatography and spectrophotometry of each fraction showed marked differences in PD distribution in collagen from normal and pathologic bones. The amount of cross-linked peptide containing PD was greater in AO and vitamin D-deficient bone collagen than in normal collagen. The differences in PD distribution may result from differences in degree of lysine hydroxylation. (16 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Anderer FA 31
Anzano MA 22
Asseo PP 19
Azar HA 13
Banes AJ 46
Barazzone P 6
Battle J 17
Baumann H 36
Becker JH 45
Bellingham AJ 5
Bhavanandan VP 30
Blankenship DT 39
Borrasca AL 17
Boutwell RK 9
Bova R 28
Brundage R 14
Busch H 1
Busch RK 1
Caras IW 43
Chlopkiewicz B 41
Clark CM 11
Clark RB 34
Clift SM 10
Clift SM 43
Cohn SH 40
Comolli R 35
Conradt P 32
Contieri E 28
Cook JS 45
Cortes E 40
Costa J 13
Davidson EA 30
De Brabander M 38
De Mey J 38
Devitt P 18
Doi Y 21
Dorrington KJ 12
Elliott MS 44
Ellis KJ 40
Emura M 32
Eriksson S 10
Eriksson S 43
Franke WW 6
Freisheim JH 39
Fridovich SE 27
Friedman JA 34
Friedrich E 29
Froussard P 2
Fukuda M 37
Gabbiani G 6
Garbisa S 14
Gartenhaus W 40
Gersten DM 23
Geuens G 38
Goldfarb RH 14
Goodlad GA 11
Grant AG 42
Grant DA 42
Green DA 34
Gudas LJ 10
Gudas LJ 43
Hagmann W 31
Hanefeld F 15
Hayashi H 33
Hearing VJ 23
Held WA 36
Hernandez F 17
Hobbs DS 16
Hofmann T 12
Horne C 12
Hotate T 21
Ishida Y 8
Ishimaru Y 33
Ishizeki J 8
Jaroffke B 15
Kamata N 21
Kapanci Y 6
Kataura A 21
Katz SI 24
Keenan JP 5
Kirley SD 4
Klein M 12
Knowles BB 27
Koga Y 33
Koide H 26
Kolb H 29
Koziorowska J 41
Kuboki Y 46
Kumar AA 39
Kung H 16
Lai CY 16
Lamb LC 22
Lanzer WL 13
Levy SB 2
Lin CW 4
Liotta LA 13
Liotta LA 14
Lodish HF 27
Lonovics J 18
Luger TA 24
Maj S 41
Mangum JH 39
Marchalonis JJ 23
Martin DW 10
Martin DW 43
Matthei S 32
Matthews RH 44
McNutt NS 7
Mechanic GL 46
Minton JP 44
Nishio M 21
Nola E 28
Novello E 20
Nuydens R 38
O'Grady RL 25
Ohno J 26
Oppenheim JJ 24
Panfili E 20
Papaleo G 28
Pestka S 16
Pfeifle J 31
Puca GA 28
Rai K 40
Rayford PL 18
Rheinwald JG 3
Riboni L 35
Roberts AB 22
Russeli NH 5
Sakurai T 21
Salmon J 5
San Miguel J 17
Savvidou G 12
Sawitsky A 40
Schlepper-Schaf 29
Schwartz AL 27
Scrobogna S 20
Sica V 28
Siegel GP 14
Siegert M 15
Siemes H 15
Smith JM 22
Soeda N 26
Sporn MB 22
StPierre M 4
Stadler BM 24
Staehelin T 16
Stephens RW 25
Takeuchi T 21
Tautt J 41
Tempelis LD 2
Terranova V 14
Thompson JC 18
Tokuda S 33
Torre R 20
Tracopoulos GD 19
Trani S 20
Uchida M 21
Ullman B 10
Ullman B 43
Umemoto J 30
Upfold LI 25
Vartsky D 40
Vaswani A 40
Verma AK 9
Vicente V 17
Vriz G 20
Watson LC 18
Willebrords R 38
Wu YJ 3
Yamauchi M 46
Yasumura S 40
Yee C 13
Zand R 44
Zanzi I 40

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In this issue:

- **NUCLEOPROTEINS** 1-9
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** 10-16
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** 17-32
- **BINDING/ RECEPTOR PROTEINS** 33-37
- **MEMBRANE/ SURFACE PROTEINS** 38-48
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** 49-50
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** 51-56
- **POLYAMINES - SYNTHESIS AND METABOLISM** 57-58
- **OTHER RELATED STUDIES** 59-62

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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NUCLEOPROTEINS

1. LOSS OF CHROMOSOMAL HIGH MOBILITY GROUP PROTEINS HMG1 AND HMG2 WHEN MOUSE NEUROBLASTOMA AND FRIEND ERYTHROLEUKEMIA CELLS BECOME COMMITTED TO DIFFERENTIATION.

Seyedin SM, Pehrson JR, Cole RD
Dept. Biochemistry, Univ. California, Berkeley, CA, 94720
Proc Natl Acad Sci USA; 78(10):5988-5992 1981

Chromosomal high mobility group (HMG) proteins HMG1 and HMG2 from mouse neuroblastoma cells and Friend erythroleukemic cells were analyzed by acetic acid/urea/polyacrylamide gel electrophoresis. Compared to rapidly growing cells, levels of HMG1 and HMG2 were decreased in mouse neuroblastoma cells that had been induced to differentiate by serum deprivation. This comparison revealed a reciprocal relationship between these HMG proteins and H10, a histone known to be in higher concentrations in nondividing cells. When cell growth was inhibited by means of density inhibition, however, HMG1 and-2 levels were not affected in either HeLa or mouse neuroblastoma cells, even though H10 did accumulate. This observation establishes that HMG1 and-2 contents are not correlated with mitotic rate per se. Treatment of mouse neuroblastoma by sodium butyrate, which stops cell division without commitment to differentiation, had no effect on the level of HMG1 and-2. However, the level was decreased by dibutyryl cyclic AMP and dimethyl sulfoxide treatments, which, like serum deprivation, induced irreversible morphological differentiation in the neuroblastoma cells. Moreover, induction of differentiation (Hb synthesis) in Friend erythroleukemic cells by dimethyl sulfoxide showed a decrease in the contents of HMG1 and-2. These observations suggest that preferential loss of HMG1 and-2 in mouse neuroblastoma and Friend erythroleukemia cells may be related to commitment of these cells to differentiation. (Author abstract) (32 Refs)

2. DETECTION OF A HISTONE 2A RELATED POLYPEPTIDE IN DIFFERENTIATED HUMAN MYELOID CELLS (HL60) AND ITS DISTRIBUTION IN HUMAN ACUTE LEUKEMIA (MEETING ABSTRACT).

Sarin PS, Pantazis P, Gallo RC
No affiliation given
Tenth International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. 187 pp., 1981.

A human myeloid cell line, HL60, has been shown to terminally differentiate to mature granulocytes in the presence of dimethylsulfoxide (DMSO) and other compounds. A histone polypeptide (HP) of an apparent mol wt of 12,500 has been identified among the acid extractable chromosomal proteins of DMSO treated HL60 cells. The detection of HP in total chromatin, total cell proteins or acid extracts of intact HL60 cells treated with DMSO indicates that HP is present in intact cells and is not generated during the isolation procedures used for preparation of chromatin or histones. No radiolabeled HP was detected when chromatin was isolated from a mixture of radiolabeled mouse cells and HL60 cells treated with DMSO. The relative increase in the amount of HP in DMSO treated HL60 cells correlates with a concomitant decrease of histone H2A. Fingerprint analysis of tryptic digests shows HP to be related to histone H2A. Generation of HP from endogenous and exogenous H2A is probably due to a proteolytic enzyme associated with chromatin of differentiated HL60 cells. HP was not observed in cell lines derived from leukemic lymphoblasts (CCRF-CEM, MOLT-4), human leukemic erythroblasts (K562), mouse leukemic erythroblasts (friend cells), normal human liver, normal mouse liver and spleen. Low levels of HP were detected in human lymphocytes and granulocytes from peripheral blood of normal donors. High levels of HP equivalent to those observed in DMSO treated HL60 cells were detected in WBC from patients with acute lymphoblastic leukemia (4/4), and acute myelogenous leukemia (6/6), but HP was absent in WBC from patients with chronic lymphocytic leukemia (3/3) and chronic myelogenous leukemia (5/5). Our results suggest that: (a) HP is a proteolytic product of histone H2A and not a product of an independent gene; (b) the observed level of HP in HL60 cells is related to the stage of differentiation; and (c) HP is a potential biological marker for identification and classification of human acute leukemia. (no Refs)

3. NONHISTONE PROTEINS IN MOUSE TERATOCARCINOMAS (MEETING ABSTRACT).

Bluthmann K, Illmensee K
Department de Biologie Animale, Universite de Geneve, CH-1224 Geneve, Switzerland
Experientia; 37(6):638 1981

Mouse teratocarcinomas with restricted capacities for differentiation are especially suitable to investigation at the molecular level the developmental processes from a stem cell to a particular cellular phenotype. The gradual acquisition of cells for specialized functions is presumably achieved by differential regulation of gene activities at the chromosomal level. There is convincing evidence that nonhistone proteins (NHPs) are involved in this process. We have isolated them from 5 unidifferentiated teratocarcinomas as well as from a multidifferentiated and an undifferentiated type. Separation of the NHPs by differential salt extraction and hydroxyapatite chromatography gives 3 fractions: tris-soluble 0.35 M NaCl-extractable, and 2 M NaCl/5 M urea-extracted NHPs. Their comparison by gel-electrophoresis reveals that there are differentiation-specific NHP-patterns which are characteristic for a special cellular phenotype. We intend to test the biological activity and specificity of the NHP-fractions via microinjection into teratocarcinoma cells. (no Refs)

4. THE EFFECT OF ADDED H1 HISTONE AND POLYLYSINE ON DNA SYNTHESIS AND CELL DIVISION OF CULTURED MAMMALIAN CELLS.

Kundahl E, Richman R, Flickinger RA
Dept. Biological Sciences, State Univ. New York at Buffalo, Buffalo, NY, 14260
J Cell Physiol; 108(3):291-298 1981

The effect of adding whole histone, various histone fractions, polylysine, and polyarginine on the rate of cell division and DNA synthesis of three murine cell lines (cultured Friend erythroleukemia cells and two mouse lymphoma cell lines, el-4 and S-49) was studied. Low levels (10 ug/ml) of H1 histone or polylysine stimulated DNA synthesis by the creation of additional initiation sites for replication and production of smaller av replicon sizes. Untreated Friend erythroleukemia cells underwent a decrease in cell division rate on days 2-5 of culture while the decrease in rate of cell division was much less for cells treated with H1 histone or polylysine. This might mean that the observed results are not due to the stimulation of cell division but rather to a continuation of cell division in the H1 and polylysine-treated cultures. However, the data from the experiments with the lymphoma cells showed clearly that H1 histones can stimulate the rate of cell division before the rate of cell division of the untreated cells began to plateau. In the lymphomas, there was a stimulation of the rate of cell division after 1 day of cultre and a slight stimulation of cell division of the Friend cells after 2 days of culture. These data indicated that there is not a long lag before H1 histone and polylysine affect cell division and that these compounds are not acting merely by affecting the level of depletion of some material in the culture medium after a prolonged period of culture. DNA fiber autoradiographic experiments revealed that replicon size is decreased in the cells cultured with H1 histone and polylysine at later periods of culture. (25 Refs)

5. IDENTIFICATION OF A POLYPEPTIDE COMPONENT OF MOUSE MYELOMA DNA POLYMERASE GAMMA.

Matsukage A, Tanabe K, Yamaguchi M, Taguchi YN, Nishizawa M, Takahashi T, Takahashi T
Lab. Biochemistry, Aichi Cancer Center Res. Inst., Chikusa-ku, Nagoya 464, Japan
Biochim Biophys Acta; 655(3):269-277 1981

Mouse myeloma DNA polymerase gamma was extensively purified to a final specific activity of 156,000 units (nmol dTMP incorporation per hr) per mg protein on (rA)n.(dT)12-18 as a template primer. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of protein fractions obtained by DNA-cellulose column chromatography revealed that the amount of a polypeptide of Mr = 47,000 changed proportionally with DNA polymerase gamma activity. A minor polypeptide of Mr = 140,000 also seemed to change with the enzyme activity, but other polypeptides did not. Analysis by 125I-labeled peptide mapping indicates that the Mr 47,000 polypeptide in the mouse myeloma DNA polymerase gamma preparation is structurally related to the Mr 47,000 polypeptide of chick embryo DNA polymerase gamma. An antibody against chick embryo DNA polymerase gamma cross-reacted with the mouse enzyme, indicating a structural relationship between avian and murine enzymes. Since the Mr 47,000 polypeptide accounts for 31.4% of total protein in the purified preparation, the specific activity is estimated to be about 490,000 units per mg of the Mr 47,000 polypeptide. The rate of poly(dT) elongation by the purified enzyme was 1,260 nucleotides per min. This value is in the same range as the turnover number (1,530 nucleotides per min per enzyme molecule) which is calculated from the 'expected' specific activity with respect to the Mr 47,000 polypeptide and the mol wt (Mr = 188,000 on the assumption of a tetrameric structure of the Mr 47,000 polypeptide). Results indicate that the Mr 47,000 polypeptide is a component of the mouse myeloma DNA polymerase gamma. (Author abstract) (19 Refs)

6. LOCALIZATION OF PHOSPHOPROTEIN C23 IN NUCLEOLI BY IMMUNOLOGICAL METHODS.

Olson MO, Guetzow K, Busch H
Dept. Biochemistry, Univ. Mississippi Medical Center, Jackson, MS, 39216
Exp Cell Res; 135(2):259-265 1981

Antiserum to a major phosphorylated nucleolar protein, C23 (mol wt 103,000, pI 5.2) from Novikoff hepatoma was produced in rabbits. By immunodiffusion analysis, the antiserum produced precipitin bands and with various crude extracts of nucleoli, but not with extranucleolar or cytosol fractions. The specificity of the antibody was assessed using acid-urea polyacrylamide gel electropherograms of acid-soluble nucleolar proteins in which the separated proteins were transferred to nitrocellulose sheets. The purified antibody reacted predominantly with protein C23 as visualized by the immunoperoxidase procedure. By the indirect immunofluorescence technique, protein C23 was localized predominantly to nucleoli of Novikoff hepatoma or normal rat liver cells. In Novikoff hepatoma cells, traces of fluorescence were seen near the inner layer of the nuclear envelope. Additional narrow regions of fluorescence extended from the nucleoli into the extranucleolar areas of some Novikoff cells. The nucleolar areas of fluorescence were smaller but brighter in the normal liver than in Novikoff hepatoma, consistent with the small size of rat liver nucleoli. These data indicate that the major location of protein C23 is the nucleolus. (Author abstract) (33 Refs)

7. LOCALIZATION OF DNA METHYLTRANSFERASE IN THE CHROMATIN OF FRIEND ERYTHROLEUKEMIA CELLS.

Creusot F, Christman J K
Dept. Biochemistry, Mount Sinai Medical Center, 1 East 100th St., New York, NY, 10029
Nucleic Acids Res; 9(20):5359-5381 1981

Chromatin fragments released from intact Friend erythroleukemia cell nuclei during limited incubation with micrococcal nuclease, DNase II or DNase I were analyzed to determine the distribution of DNA methyltransferase in chromatin. The enzyme was released in a free form when internucleosomal DNA was digested with micrococcal nuclease but was found associated with Mg++-precipitable polynucleosomes after DNase II digestion. Less than 25% of the enzyme was released from nuclei incubated with DNase I under conditions where transcriptionally active chromatin should have been completely digested. These results indicated that the bulk of DNA methyltransferase was bound to 'linker' DNA in condensed regions of chromatin. Preferential rebinding of free enzyme to linker DNA was also demonstrated in vitro. The possibility that chromatin proteins play a role in regulating access of DNA methyltransferase to specific sites in DNA is discussed in light of these findings. (Author abstract) (38 Refs)

8. ACTIVATION OF PURIFIED HEPATOMA RNA POLYMERASE I BY HOMOLOGOUS PROTEIN KINASE NII.

Duceman BW, Rose KM, Jacob ST
Dept. Human Genetics, Yale Univ. Coll. Medicine, New Haven, CT, 06510
J Biol Chem; 256(21):10755-10758 1981

We have recently purified a cyclic nucleotide-independent, heparin-sensitive nuclear protein kinase (NII) from Morris hepatoma 3924A and demonstrated an apparent relationship of this kinase to the two subunits (Mr = 42,000 and 24,600) of RNA polymerase I. When homogeneous protein kinase NII was recombined with purified homologous RNA polymerase I containing limiting quantities of endogenous kinase, RNA synthesis was stimulated as much as five-fold during a 90-min incubation. The enhanced RNA synthesis was due to an increase in the av RNA chain length; protein kinase did not alter the number of RNA molecules synthesized by the polymerase. Phosphorylation of RNA polymerase occurred at serine and threonine moieties. Unlike the NII kinase, purified homologous NI kinase did not phosphorylate RNA polymerase I and, as a result, did not alter transcription. These data indicate that (1) RNA polymerase I is activated by protein kinase NII, (2) endogenous protein kinase NII remaining with highly purified RNA polymerase I does not fully phosphorylate RNA polymerase I in vitro, and (3) protein kinase NII is capable of regulating RNA polymerase I activity by preventing premature termination of RNA chains. (Author abstract) (21 Refs)

9. LOCALIZATION OF PHOSPHOPROTEIN C23 TO NUCLEOLAR STRUCTURES AND TO THE NUCLEOLUS ORGANIZER REGIONS.

Lischwe MA, Richards RL, Busch RK, Busch H
Dept. Pharmacology, Baylor Coll. Medicine, Houston, TX, 77030
Exp Cell Res; 136(1):101-109 1981

After extraction of Novikoff hepatoma nucleoli with 4 M urea/3 M LiCl, phosphoprotein C23 was isolated by DEAE-cellulose and Bio-Rad AG3-X4A column chromatography. Immunization of rabbits with the highly purified protein C23 resulted in the production of a specific antibody as determined by Ouchterlony diffusion analysis. When the immunoperoxidase method was used to localize protein C23 in cells, it was found in 'fibrillar centers' (nucleolonemas) in nucleoli. Protein C23 was also demonstrated to be present on the nucleolus organizer regions (NORs) of metaphase chromosomes. (Author abstract) (33 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

10. HUMAN CELLS CONTAIN TWO FORMS OF PP60C-SRC.

Shealy DJ, Erikson RL
Dept. Pathology, Univ. Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO, 80262
Nature; 293(5834):666-669 1981

The presence of normal cell protein pp60c-src in normal human fibroblasts and A4 31 human epidermoid carcinoma cells was studied using electrophoresis with a 10% polyacrylamide gel. A431 cells could be resolved into two bands. Both forms were phosphorylated with mol wt of approx 60,000 and 59,000. Normal human fibroblasts also contained two forms of pp60c-src, both of which were phosphorylated; about 1/3 of the pp60c-src was of the higher mol wt and 2/3 of the lower. To confirm that the two proteins immunoprecipitated from A431 cells were actually pp60c-src, limited V-8 protease digests of the forms were compared with authentic PP60c-src immunoprecipitated from chick embryo fibroblasts. Phosphoamino acid analysis of the A431 pp60c-src indicated that the 60,000 mol wt form contained about 70% phosphotyrosine and 30% phosphoserine, and the 59,000 mol wt form 99% phosphoserine and only 1% phosphotyrosine. Thus according to both phosphoamino acid analysis and limited V-8 digestion, each form was phosphorylated predominantly at either tyrosine (60,000 mol wt) or serine (59,000 mol wt) residues. 35S-methionine chymotryptic maps showed that the two pp60c-src forms are clearly related, although several significant differences were observed. Possible explanations for the existence of two forms of pp60c-src in human cells are discussed. (20 Refs)

11. MODIFIED PROTEIN KINASE ACTIVITY AND ISOZYME DISTRIBUTION IN ADENOCARCINOMA OF THE HUMAN COLON.

Raul F, Launay J-F, Galluser M, Doffoel M, Marescaux J, Grenier JF
Unite 61, INSERM, Ave. Moliere, Strasbourg Hautepierre, France
Biomedicine [Express]; 35(3):98-100 1981

Protein kinase activity and histone kinase isozyme distribution have been determined in soluble extracts of adenocarcinoma of the human colon and compared to adjacent normal mucosa. The results show an enhancement in endogenous protein kinase activity and the presence of an additional isozyme (PKI) for histone kinase activity in the tumor tissue. PKI activity exhibited a peculiar behavior in comparison to the isozyme PKII present in both carcinoma and normal mucosa after dialysis of the soluble extracts. It is suggested that alteration of intracellular regulatory processes involved in PKI activity might be related to the maintenance of the proliferate state in human colon carcinoma. (Author abstract) (16 Refs)

12. ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL LOCALIZATION OF MYOSIN IN CULTURED FIBROBLASTIC CELLS.

Willingham MC, Yamada SS, Bechtel PJ, Rutherford AV, Pastan IH
Lab. Molecular Biology, NCI, NIH, Bethesda, MD, 20205
J Histochem Cytochem; 29(11):1289-1301 1981

Nonmuscle myosin in the cytoplasm of cultured fibroblastic cells has been localized using light and electron microscopic immunocytochemistry. Antibodies to purified fibroblast myosin were produced in goat and rabbit and purified by affinity chromatography. Light microscopic immunofluorescence lo calization showed patterns similar to those previously published. Electron microscopic localization using the ethyldimethyl aminopropyl carbodiimide-glutaraldehyde-saponin (EGS) fixation-permeabilization procedure and the ferritin bridge localization method produced quantifiable localization in intracellular sites with well-preserved ultrastructural morphology. Myosin was found to be a major component of the cytosol. It was distributed diffusely with no preferential localization on membranous organelles. Myosin was found to be slightly concentrated on the surface of microfilament-containing structures, including the subplasmalemmal microfilament mat stress fibers, occasionally with an interrupted periodicity. However, no myosin was found in surface ruffles or microvilli. Morphometric quantitation showed that the majority of the cell's myosin was in the cytosol. This location is compatible with myosin being a component of the microtrabecular lattice of the cytoplasmic ground substance. The concentration of myosin in association with microfilaments was only twice that of the cytosol. This interpretation must be somewhat tempered by the possibility that some myosin bound to tightly packed actin may be inaccessible. The significance of this distribution of myosin in cell function is discussed. (Author abstract) (38 Refs)

13. THE DISTRIBUTION OF KERATIN TYPE INTERMEDIATE FILAMENTS IN HUMAN BREAST CANCER: AN IMMUNOHISTOLOGICAL STUDY.

Altmannsberger M, Osborn M, Holscher A, Schauer A, Weber K
Dept. Pathology, Univ. Gottingen, D-3400 Gottingen, W. Germany
Virchows Arch [Cell Pathol]; 37(3):277-284 1981

Antibodies to different intermediate filament proteins can be used to distinguish cells of epithelial, mesenchymal, muscle, glial and neuronal origin. Antibodies to prekeratin which characterize cells of epithelial origin, and antibodies to vimentin which recognize cells of mesenchymal origin have been used to study 20 cases of breast carcinoma (16 infiltrating ductal carcinomas and 4 infiltrating intraductal carcinomas), 2 cases of cystic breast disease, 2 fibroadenomas and 1 case of benign cystosarcoma phylloides. The prekeratin and vimentin were detected using specific antibodies to these proteins by immunofluorescence microscopy using alcohol fixed paraffin-embedded tissues. In eighteen out of the 20 carcinomas the tumor cells were strongly and specifically stained by antibodies to prekeratin. Different tumors gave different patterns of prekeratin staining. In contrast, when the same specimens were tested with the vimentin antibody, the tumor cells were unstained, and instead only the usual strong staining to fibroblasts and blood vessels in the stroma was observed. In cystic breast disease, fibroadenomas, and benign cystosarcoma phylloides, cells of epithelial origin were strongly stained by the prekeratin but not by th e vimentin antibody. (Author abstract) (21 Refs)

14. PURIFICATION OF A NEW PROTEIN STIMULATING RNA POLYMERASE II FROM EHRLICH ASCITES TUMOR CELLS: COMPARISON WITH PROTEINS PURIFIED BEFORE.

Nakanishi Y, Sekimizu K, Tamura H, Natori S
Faculty Pharmaceutical Sciences, Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan
J Biochem (Tokyo); 90(3):805-814 1981

A protein factor named S-I(b) that stimulates the activity of RNA polymerase II was purified from Ehrlich ascites tumor cells. The final preparation gave a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This protein has a mol wt of 24,000 and is significantly smaller than S-II and S-II', stimulatory proteins of RNA polymerase II purified previously. However, S-I(b) cross-reacted with antibody against S-II and its peptide map obtained after radioiodination was identical with that of S-II' (a phosphorylated form of S-II), indicating that S-I(b) and S-II share a common primary structure. S-I(b) is suggested not to be a degradation product of S-II produced during the purification process, and the structural relations between S-II and S-II' are discussed. (Author abstract) (35 Refs)

15. A COMPARISON OF PURIFIED POLY(ADP-RIBOSE) POLYMERASES FROM EHRLICH ASCITES TUMOR CELLS, PIG THYMUS, AND HELA S3 CELLS.

Holtlund J, Kristensen T, Ostvold AC, Laland SG
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Eur J Biochem; 119(1):23-29 1981

Poly(ADP-ribose) polymerases from Ehrlich ascites tumor cells, pig thymus, and HeLa S3 cells were purified by chromatography on DNA-agarose and Blue Sepharose. A molecular mass of 112,000 Da was found for all three polymerases. Fragmentation of polyacrylamide-gel-embedded polymerases with cyanogen bromide, and subsequent analysis of the fragments by polyacrylamide gradient gel electrophoresis, showed great similarities with regard to fragment sizes. The amino acid composition of the pig thymus enzyme was very similar to that of the polymerase from Ehrlich ascites tumor cells, and the terminal amino group appeared to be blocked. The HeLa polymerase electrofocused in two peaks at pH 8.8 and 5.5, while the Ehrlich ascites tumor cell and the pig thymus enzyme focused in single peaks at pH 9.4 and 9.6, respectively. Removal of residual DNA by treatment with hydroxyapatite abolished these differences in apparent isoelectric points; all three polymerases focused at pH 9.8. No important differences were found with regard to the effect of a number of substances on the synthesis of poly(ADP-ribose). Apparent Michaelis constants for NAD of 41 uM, 48 uM, and 34 uM were found for polymerase from Ehrlich ascites tumor cells, pig thymus, and HeLa S3 cells, respectively. All these results indicate that the three polymerases, which represented the major poly(ADP-ribose) polymerase activity in the organisms investigated, are closely related proteins. (Author abstract) (25 Refs)

16. ONCOMODULIN - A TUMOUR SPECIFIC MR 11500 CALCIUM BINDING PROTEIN OF WIDE DISTRIBUTION (MEETING ABSTRACT).

MacManus JP, Whitfield JF
Division of Biological Sciences, National Research Council of Canada, Ottawa, K1A OR6, Canada
Oncodev Biol Med; AbstractsBook,1:57 1981

An acidic Mr 11500 calcium binding protein (now termed 'oncomodulin') has been located chemically and immunologically in rodent hepatomas and sarcomas. This protein cannot be located in any normal rat tissue either adult or fetal. Radioimmunoassay indicated that if present in normal tissue, the concentration of oncomodulin must be less than 1/20,000th that of tumor tissue. Oncomodulin although having similar amino acid composition to known calcium binding proteins is not parvalbumin or calmodulin. Tryptic peptide analysis also show oncomodulin not to be a fragment of calmodulin or troponin C. However oncomodulin can substitute for calmodulin in activating calmodulin dependent enzymes and in the initiation of DNA synthesis in calcium deprived cells. Preliminary results indicate the presence of oncomodulin in a wide variety of human tumor cells. The appearance of this putative tumor specific modulator protein will be discussed from a diagnostic viewpoint, and also in relation to perturbations in growth control by calcium: calmodulin of neoplastic cells. (no Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

17. AMINO ACID SEQUENCE OF A HUMAN LEUKOCYTE INTERFERON.

Levy WP, Rubinstein M, Shively J, Del Valle U, Lai CY, Moschera J, Brink L, Gerber L, Stein S, Pestka S
Roche Inst. Molecular Biology, Nutley, NJ, 07110
Proc Natl Acad Sci USA; 78(10):6186-6190 1981

The primary structures of three major species of human leukocyte interferon differ from the structure predicted from the DNA sequence of recombinants containing leukocyte interferon-coding regions. Compared to the recombinant interferon produced in bacteria, three of the purified natural proteins isolated from WBC lack the 10 COOH-terminal amino acids suggested by the DNA sequence. (Author abstract) (36 Refs)

18. PLASMA INTER-ALPHA TRYPSIN INHIBITOR (IATI) IN METASTATIC BREAST CANCER (MEETING ABSTRACT).

Chawla RK, Miller FW, Lawson DH, Richmond A, Nixon DW, Rudman D
Department of Medicine, Emory University, Atlanta, GA, 30322
Oncodev Biol Med; AbstractsBook,1:P21 1981

We had previously shown the prevalence of low mol wt proteinuria (Mr less than 60 K) in most patients with metastatic cancers. A family of 5 new glycoproteins was subsequently characterized from different cancer urines. Of these, EDC1 (Mr 27.5 K) accounted for 50-75% of the total urinary proteins. It inhibited trypsin and chymotrypsin and was antigenically related to a normal plasma protein IATI, Mr 160 K. Urinary EDC1 levels (mg/g creatinine) were measured in a) normal healthy controls, b) metastatic breast cancer patients, c) non-cancer patients, and d) preoperative patients with localized breast mass. Postoperative followup showed a marked decline in EDC1 following removal of tumor. The IATI-plasma profile on gel-filtration, showed two forms of IATI in healthy controls, one at Mr 160 K and the other at Mr 55 K. The second peak was invariably absent in heavy excretors of EDC1. The total immunoreactive IATI in the metastatic breast cancer group was about 2/3 of that in healthy controls. When purified IATI was incubated with trypsin, an antitryptic molecule (Mr 27K) was formed which cross-reacted with antisera to EDC1 and IATI and had the physico-chemical characteristics of EDC1. We postulate that plasma IATI mediates and inhibits tumor-associated proteolytic activity and is eventually degraded to EDC1 which is rapidly cleared by kidneys. (no Refs)

19. CHARACTERIZATION OF HUMAN HYALURONECTIN IN GRAFTED NUDE MICE TUMOURS (MEETING ABSTRACT).

Girard N, Chauzy C, Olivier A, Delpech B
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Oncodev Biol Med; AbstractsBook,1:P18 1981

Hyaluronectin is a glycoprotein purified from human brain on the basis of its hyaluronic acid high affinity. It was also found in tumors as a marker of mesenchymal components. It was seen in primary tumors likewise in heterotransplanted human tumor in nude mice. Ten cultured human tumor cell lines were inoculated into nude mice and produced tumors. The histological pattern correlated with that of the primary tumors. Hyaluronectin was found in all grafted tumors. A species specificity of hyaluronectin could be defined by absorption of the anti-human brain hyaluronectin antiserum and immunohistological techniques. Thus human determinants were detected when a mouse antigen absorbed antiserum was used. Hyaluronectin was seen in the intercellular substance of the entire tumor in grafts of 2 human glioma cell lines. In these grafts the antigen was of the human type. In all other grafts derived from either nervous or non nervous tumor cultures, hyaluronectin was located in the connective stroma reaction and was of the mouse type. (no Refs)

20. SYNTHESIS AND TURNOVER OF BASEMENT MEMBRANE COMPONENTS BY MOUSE EMBRYONAL CARCINOMA CELLS IN SERUM-FREE, HORMONE-SUPPLEMENTED MEDIUM (MEETING ABSTRACT).

Salomon DS, Liotta LA, Foidart JM, Yaar M
Laboratory of Pathophysiology, National Cancer Institute, Bethesda, MD
Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Using specific antibodies to type IV collagen, laminin and fibronectin, cytoplasmic and extracellular staining was observed in and around cells which resemble endoderm (END) cells. Furthermore, some of these cells stained with type I collagen antibody. Immunoassay of the culture medium revealed that cells were secreting type IV collagen, laminin and fibronectin. Incorporation of labelled proline into bacterial collagenase-sensitive proteins accounted for 2-4% of the newly synthesized proteins of which over 90% was found in the culture medium. Electrophorectic analysis showed that the cells were synthesizing and secreting collagenase-sensitive peptides resembling type IV collagen and to a lesser extent alpha1 and alpha2 chains of type I collagen. Treatment of the cultures with retinoic acid promoted EC cell differentiation into END cells as evidenced by the appearance of plasminogen activator. Retinoid-derived END cells exhibited an increase in type IV collagen production, which constituted approx 10-15% of the total secreted proteins. Little or no detectable type I collagen was found under these conditions. Concomitant with the appearance of plasminogen activator, a neutral protease was found in the culture medium which was capable of degrading type IV collagen. The collagenase which was produced by END cells was sensitive to protease activation (trypsin or plasmin) suggesting that part of the activity was in a latent form in the culture medium. Plasmin was also capable of degrading the newly synthesized laminin to specific peptides. (no Refs)

21. SERUM SPREADING FACTOR: EFFECTS ON RF1 RAT OVARY CELLS AND 1003 MOUSE EMBRYONAL CARCINOMA CELLS IN SERUM-FREE MEDIA (MEETING ABSTRACT).

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Department of Biology Science, University of Pittsburgh, Pittsburgh, PA, 15260

Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Serum spreading factor (SSF), a glycoprotein partially purified from human serum, influences the growth and morphology of a number of fibroblastic and epithelial cell types in serum-free media (SFM). The major portion of the protein in the SSF preparations is estimated to be a mol wt between 60,000 and 90,000 by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) of reduced samples. SSF is similar to fibronectin or cold-insoluble globulin (CIg) in that it shows an affinity for the plastic cell culture substratum, but appears to be distinct from CIg by several criteria. Previously CIg at 0.5 to 5 ug/ml was shown to mediate cytokinesis and growth of RF1 rat ovary cells in SFM supplemented with insulin (I), transferrin (TF) and hydrocortisone. Similar results are obtained if CIg is replaced with SSF preparations in the same concentration range. The 1003 mouse embryonal carcinoma cell line is a multipotential line which differentiates predominately into neurons in SFM. Addition of 5 ug/ml SSF to 100 3 cells in SFM supplemented with I, TF and CIg results in increased saturation density, increased spreading of islets of cells on the dish and delayed differentiation compared to cells in the absence of SSF. In the absence of CIg, 1003 cells do not attach to the dish in the presence of SSF. (2 Refs)

22. COLLAGEN AND COLLAGEN-DERIVED FRAGMENTS ARE CHEMOTACTIC FOR TUMOR CELLS.

Mundy GR, DeMartino S, Rowe DW

Dept. Medicine, Univ. Texas Health Science Center, San Antonio, TX, 78284

J Clin Invest; 68(4):1102-1105 1981

Organs that are rich in collagen such as liver, lungs, and bone are frequently sites of tumor cell metastasis. In this study, we have found that cultured tumor cells of human and rat origin migrated unidirectionally in response to collagen in vitro. Synthetic di- and tri-peptides that contained amino acid sequences found frequently in the collagen helix caused similar effects. These results are consistent with the hypothesis that collagen or collagen fragments released during connective tissue remodeling may be important in tumor cell metastasis. (Author abstract) (14 Refs)

23. NATURAL ANTIBODIES AGAINST TUBULIN, ACTIN, MYOGLOBIN, THYROGLOBULIN, FETUIN, ALBUMIN AND TRANSFERRIN ARE PRESENT IN NORMAL HUMAN SERA AND MONOCLONAL IMMUNOGLOBULINS FROM MULTIPLE MYELOMA AND WALDENSTROM'S MACROGLOBULINEMIA MAY EXPRESS SIMILAR ANTIBODY SPECIFICITIES.

Avrameas S, Guilbert B, Dighiero G
Unite d'Immunocytochimie, Institut Pasteur, Paris, France
Ann Immunol (Paris); 132(2):231-236 1981

Natural antibodies and their relationship with autoantibodies were systematically studied. Seven different antigens (tubulin, thyroglobulin, actin, myoglobin, fetuin, albumin, and transferrin) were immobilized on Ultragel beads and these immunoadsorbents were used for the isolation of natural antibodies in sera from a pool of 800 healthy donors and from three healthy individuals. The presence of natural antibody-like function was also studied in 62 patients with multiple myeloma or Waldenstrom's macroglobulinemia. Natural antibodies against the antigens studied were found in normal sera. Among the 62 patients, sera from 3 reacted strongly with actin and sera from 1 reacted strongly with tubulin. The anti-tubulin antibodies were found to react only with immobilized tubulin and this reaction was only inhibited by tubulin. The three anti-actin antibodies reacted mainly with immobilized actin but also with immobilized tubulin and thyroglobulin. The reaction between anti-actin antibodies and immobilized actin was inhibited mainly by actin but also by tubulin and thyroglobulin. The results obtained with sera from patients carrying monoclonal immunoglobulins and showing antibody specificty close to that observed with natural antibodies indicated, that at least for some patients, the monoclonal immunoglobulin produced may represent the expansion of a clone producing a natural antibody, directed in some instances, against an auto-antigen. (8 Refs)

24. BASEMENT MEMBRANE CHANGES IN BREAST CANCER DETECTED BY IMMUNOHISTOCHEMICAL STAINING FOR LAMININ.

Albrechtsen R, Nielsen M, Wewer U, Engvall E, Ruoslahti E
Univ. Inst. Pathological Anatomy, Frederik V's vej 11, DK-2100 Copenhagen, Denmark
Cancer Res; 41(12):5076-5081 1981

The distribution of the basement membrane glycoprotein laminin was studied by the immunoperoxidase technique in benign and malignant human breast tissue and in axillary lymph nodes from patients with breast cancer. An antiserum prepared against rat laminin was used. The specificity of this antiserum against laminin was studied using the FL cell line of human epithelial-like cells derived from normal amniotic membrane. The antiserum reacted with these cells in immunoperoxidase staining and precipitated metabolically labeled secreted polypeptides which comigrated with polypeptides with mol wt of 400,000 and 200,000 of rat laminin in sodium dodecyl sulfate:polyacrylamide gel electrophoresis. The neoplastic cells in malignant breast tissues showed strong cytoplasmic staining for laminin, and a positive reaction was also found in lymph node metastases. In some cases in which only micrometastases were present, these cells also stained strongly for laminin. In nonmalignant breast tissues, the epithelial cells of the ducts were positive for laminin, but the staining was weaker than in the carcinomas. Pretreatment of the fixed tissue sections with trypsin markedly enhanced the staining of basement membranes for laminin. In trypsin-treated sections of normal breast tissue and benign lesions, the laminin staining delineated continuous basement membranes. In carcinomas representing the more differentiated types, basement membranes presumably produced by the tumor cells could be revealed by laminin staining, but they were thinner and discontinuous. The poorly differentiated carcinomas lacked organized basement membranes detectable by laminin staining. Our studies suggest that staining for laminin may be a useful adjunct test for detection of micrometastases in lymph nodes. The correlation of disintegration of the laminin-containing basement membranes of tumors with increasingly anaplastic appearance supports the notion that basement membranes may play a role in tumor invasion. (Author abstract) (33 Refs)

25. MOUSE MYELOMA PROTEINS WITH LAMBDA2 AND LAMBDA3 LIGHT CHAINS.

Cotner T, Blaser K, Kriedberg J, Potter M, Eisen HN
(c/o Eisen), Center Cancer Res., Massachusetts Inst. Technology, E17-128, Cambridge, MA, 02139
J Immunol; 127(5):2150-2155 1981

Serum and ascites fluid from mice bearing 260 different myeloma tumors were screened serologically for myeloma proteins having light chains like that of L315, the lambda 2 chain produced by myeloma MOPC-315. Four proteins, made by myelomas TEPC-952, CBPC-49, ABPC-72, and SAPC-15, were identified. Their light chains were essentially indistinguishable serologically from L315, and each of them also yielded the characteristic C-terminal amino acid (leucine) and C-terminal tryptic peptide of L315. However, amino acid sequences and other findings reported elsewhere have revealed that although the light chain of TEPC-952 is indeed a lambda 2 chain, the light chain of the others (CBPC-49, ABPC-72, and SAPC-15) represent another, slightly different type of light chain, which has been designated lambda 3. (Author abstract) (21 Refs)

26. DIFFERENTIATION OF HUMAN BREAST CARCINOMAS: AN IMMUNOHISTOLOGICAL STUDY OF APPROPRIATE AND INAPPROPRIATE PROTEIN PRODUCTION.

Walker RA
Dept. Pathology, Univ. Leicester, P. O. Box 65, Leicester LE2 7LX, England
J Pathol; 135(1):87-95 1981

A series of breast carcinomas was analyzed by an indirect immunoperoxidase method for evidence of production of secretory piece and beta1 pregnancy-specific glycoprotein (BPSG). Results were examined with respect to histologic differentiation and structure. Secretory piece of immunoglobulin A could be demonstrated in 29/75 carcinomas and BPSG in 28/75 tumors. No staining was evident in control sections treated with normal rabbit serum. Staining was abolished in breast carcinoma cells after absorption of anti-BPSG serum by 1.8 ug BPSG. No staining of the cells of normal breast ducts of acini occurred. More negative than positive cells were found in the section examined for both antigens. Seven of the carcinomas in which secretory piece was demonstrated had staining either in three to five discrete areas, or as single or small groups of cells in a diffuse pattern throughout the section. The other 22 tumors showed staining in one or two areas only. Findings were similar for BPSG, with 22 carcinomas having a small number of positive cells and six tumors a greater number. Poorly differentiated carcinomas had a low incidence of detection for both antigens. Well-differentiated tumors were heterogeneous in protein staining. (12 Refs)

27. STUDIES ON THE LOCALIZATION OF THE GLYCOPROTEIN GP-2 WITHIN THE RENAL GLOMERULUS IN VIVO AND IN CULTURED KIDNEY CELL STRAINS IN VITRO.

Oberley TD, Chung AE, Murphy-Ullrich JE, Mosher DF
Dept. Pathology, Univ. Wisconsin Medical Sch., Madison, WI
J Histochem Cytochem; 29(11):1237-1242 1981

Positive staining for the glycoprotein GP-2 was demonstrated in the kidney glomerulus by use of the indirect peroxidase-labeled antibody technique. At the ultrastructural level, heaviest staining for GP-2 was demonstrated along the lamina rara externa and lamina rara interna of the glomerular and tubular basement membranes, demonstrating definite molecular organization for structures which appear amorphous even at the electron microscopic level. However, GP-2 was also present in the lamina densa of the glomerular basement membrane, but not of the tubular basement membrane. The staining for GP-2 is in contrast to the predominantly mesangial staining for fibronectin. Using the indirect immunoperoxidase techniques for kidney cells cultured in vitro, it was demonstrated that cell surfaces of specific subpopulations of glomerular cells stained heavily for both fibronectin and GP-2, while renal medullary cells did not stain at all using specific antiserum to these molecules. GP-2 was present extracellularly and showed moderate staining in glomerular cell culture, while fibronectin showed heavy staining in this location. (Author abstract) (12 Refs)

28. INHIBITORY EFFECT OF HUMAN URINARY EXTRACT ON THE GROWTH OF DMBA-INDUCED MAMMARY TUMORS IN HOLTZMAN RAT.

Sarkar S, Shah PN, Bagli NP
Cancer Res. Inst., Bombay 400 012, India
Neoplasma; 28(4):457-461 1981

The effect of a crude protein fraction from human urine having gonadotropin inhibitory activity (CGIM) was observed on the growth of 7,12-dimethylbenz(a)anthracene induced mammary tumors in the Holtzman rat. Results show that while in 12 out of 13 tumors, tumor growth was retarded/regressed following 2-4 wk of CGIM treatment, tumor growth in control rats was unabated. However, no positive correlation with hormone dependency and tumor regression could be established. Further, this crude anti LH material was found to be non-toxic to tumor cells in vitro. (Author abstract) (11 Refs)

29. HUMAN MELANOMA-ASSOCIATED ANTIGENS; IMMUNOCHEMICAL AND MOLECULAR PROFILE.

Reisfeld RA, Galloway DR, Morgan AC, Walker LE, Bumol TF
Scripps Clinic and Res. Foundation, La Jolla, CA
Cancer Bull; 33(5):211-218 1981

Some of the immunochemical approaches taken in the authors' laboratory to isolate and characterize two human melanoma-associated antigens utilizing specific polyclonal and monoclonal antibodies are described. Analysis of cellular proteins released into the surrounding culture of several human melanoma cells lines indicated three major components detected by polyclonal antimelanoma xenoantisera with mol wt of 260K (260,000), 240K and 100K. The 240K component has been identified as fibronectin. The 100K component is found in spent media of both melanoma and carcinoma cell lines. The 100K and 260K dalton antigens which have affinity for specific lectins and metabolic radiolabeling with sugars strongly suggest they were glycoproteins. Other studies are reported on the use of monoclonal antibody for structural characterization, production of monoclonal antibody, subunit composition of 100K and 250K dalton melanoma antigens, and functional properties. It is apparent that the two melanoma-associated antigens of 100K and 250K dalton, defined immunochemically by monoclonal antibody, are likely to play a key role in the elicitation of host immune responses to malignant melanoma. Furthermore, it appears that changes in intracellular transport, glycosylation, and cell-surface expression of these two markers may be associated with neoplastic transformation. (12 Refs)

30. PLASMA VASOACTIVE INTESTINAL POLYPEPTIDE AND OTHER REGULATORY PEPTIDES IN CHILDREN WITH NEUROGENIC TUMOURS.

Tiedemann K, Long RG, Pritchard J, Bloom SR
(c/o Pritchard) Dept. Haematology and Oncology, Inst. Child Health and Hosp. Substitute for Sick Children, Great Ormond St., London WC1N 3JH, England
Eur J Pediatr; 137(2):147-150 1981

In consecutively diagnosed children with neurogenic tumors but no overt symptoms of vasoactive intestinal peptide (VIP) hypersecretion, VIP levels, changes in plasma VIP with tumor therapy, and other gastrointestinal regulatory peptide secretion by these tumors were examined. Four groups of children were studied prospectively: six children with newly diagnosed, untreated neurogenic tumors (Group A), 15 with previously diagnosed neurogenic tumors at various stages of treatment (Group B), 10 children with other tumors studied prior to treatment (Group C), and 12 otherwise normal children admitted for minor surgery (Group D). Of the six patients in Group A, three, including a child with intermittent diarrhea, had plasma VIP levels greater than 20 picomole/liter (pmol/l), with a mean of 25.8 +-11.5 pmol/l for the group. The latter value was not significantly different from the VIP levels of the 15 Group B children (11.1 +-1.25 pmol/l). Mean plasma VIP was significantly greater (p less than 0.05) than in Group C (mean 6.3 +-1.2 pmol/l) and Group D (mean 6.9 +-1.5 pmol/l). No significant differences were apparent between the groups in plasma levels of pancreatic polypeptide, gastrin, or pancreatic glucagon. The results suggested that plasma VIP may be useful as a diagnostic marker for childhood neurogenic tumors. (21 Refs)

31. HEPATIC RECOGNITION AND CATABOLISM OF SERUM GLYCOPROTEINS.

Ashwell G, Steer CJ
NIH, Building 10, Room 9N-105, Bethesda, MD, 20205
JAMA; 246(20):2358-2364 1981

Hepatic receptors capable of recognizing binding, and catabolizing desialylated glycoproteins are discussed. It is concluded that the hepatic receptor for galactose represents the normal physiological mechanism for maintaining serum glycoprotein homeostasis. This inference is based on evidence that asialoglycoproteins are present in the circulation and their titer is notably increased in serum of patients with hepatocellular pathology. However, the locus of desialylation is unknown. (60 Refs)

32. A FUCAN SPECIFIC LECTIN ON TERATOCARCINOMA STEM CELLS.

Grabel LB, Glabe CG, Singer MS, Martin GR, Rosen SD
Dept. Anatomy, Univ. California, San Francisco, CA, 94143
Biochem Biophys Res Commun; 102(4):1165-1171 1981

Soluble extracts of teratocarcinoma stem cells were found to agglutinate glutaraldehyde-fixed trypsinized rabbit RBC

(GTRs) to high titer. The hemagglutinin was destroyed by heating (100 C for 10 min) or by exposure to trypsin (10 ug/ ml, 60 min), indicating that it is a protein, and was inhibited by specific carbohydrates, indicating that it is a lectin. Fucoidan was a particularly potent inhibitor of both hemagglutination and of rosette formation. The carbohydrate- and erythrocyte-binding specificity of the soluble hemagglutination activity was almost identical to that of a carbohydrate-binding component (CBC) that was previously identified on the surface of mouse teratocarcinoma stem cells by the use of an erythrocyte rosetting assay. (12 Refs)

BINDING/ RECEPTOR PROTEINS

33. FC AND C3B RECEPTORS ON CULTURED RETINOBLASTOMA CELLS.

Stein PC, Rand N, Char DH
Ocular Oncology Unit, Univ. California Medical Center, San Francisco, CA, 94143
Invest Ophthalmol Vis Sci; 21(4):550-553 1981

Fc and C36 receptors were investigated to determine their presence on retinoblastoma tissue culture cells. Various techniques including radioiodination, polyacrylamide gel electrophoresis (PAGE) immunoprecipitation, affinity chromatography, and rosette assays were used to investigate tumor cells, normal human retinal pigment epithelium (RPE), and three lymphoblastoid cell lines. Results show that detergent extracts from surface labeled retinoblastoma cells bound to affinity gels prepared with human IgG, aggregated IgG, and Fc but not to control gels prepared from Fab'2 or Sepharose-4B alone. Elected cell lysate proteins were partially characterized by PAGE and autoradiography. Protein bands had mol wt of approx 4.3 x 10(4) daltons. Cultured retinoblastoma cells were found to rosette with human indicator RBC specific for C3b and Fc receptors. These results suggest that binding of serum components from retinoblastoma patients to cultured tumor cells may be mediated by Fc and C3b receptors in addition to specific binding to tumor-associated antigens. Future investigations should take steps to eliminate Fc and C3b mediated binding. (11 Refs)

34. CYCLIC AMP-BINDING PROTEINS IN HUMAN MCF-7 CELLS AND BREAST CANCER (MEETING ABSTRACT).

Eppenberger U, Handloser K, Kung W, Handschin J
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Oncodev Biol Med; AbstractsBook,1:54 1981

Several recent investigations have indicated that a relationship exists between intracellular levels of cyclicAMP (cAMP) and cAMP-dependent protein kinase isozymes Type I and Type II during normal and neoplastic growth. This study demonstrates a significant alteration in the cAMP-binding protein pattern (regulatory subunit R-II of Type II) of human neoplastic mammary tissue and human mammary MCF-7 cells as compared to normal and dysplastic mammary tissues. Fotoaffinity labeling of the cAMP receptors with 8-N3 [32P] cyclic AMP revealed on sodium dodecyl sulfate polyacrylamide gels 3 cAMP-binding proteins of R-II and a cAMP-binding protein apparent mol wt of 40,000, which could not be detected in normal and dysplastic mammary cells. It is suggested that the 40,000 mol wt protein results from a limited proteolysis. Only small amounts of the regulatory subunit R-I of isozyme Type I were observed in MCF-7 cells and breast cancer tissue, whereas in normal mammary tissue no R-I could be found. It is suggested that the cAMP-binding protein of 40,000 mol wt has some prognostic value with respect to endocrine therapy of breast cancer. (no Refs)

35. GASTRIN RECEPTORS IN HUMAN COLON CARCINOMA (MEETING ABSTRACT).

Rae-Venter B, Townsend CM, Thompson JC, Simon PM
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Gastroenterology; 80(5,part2):1256 1981

The purpose of this study was to determine whether normal or neoplastic human colons possess gastrin (GR). Partially purified plasma membranes were prepared from 8 human colon carcinoma and 3 normal colon biopsy specimens by discontinuous sucrose density gradient cetrifugation. The particulate material that collected at the 40-50% interface was used for evaluation of receptor binding. 125I-labeled synthetic human gastrin (*G-17) was prepared using EnzymoBeads (BioRad). For assay of specific gastrin receptors, 500 mg of membrane protein was incubated with 100 pM *G-17 for 2 hr at 20 C. Nonspecific binding was determined from a parallel assay in which the membranes were preincubated for 20 min at 20 C with 100 nM unlabeled G-17. Seven out of 8 of the tumor specimens had GR. The binding of *G-17 was inhibited by preincubation of the membrane with homologous peptide (100 nM), pentagastrin, caerulein and CCK-8 and 15-Leu-G-17. However, 15-Leu-G-17 was significantly less effective than 15-Met-G-17 in competing for *G-17 binding. Secretin and glucagon were noncompetitive inhibitors of *G-17 binding. The concentration of GR in 3 specimens of normal tissue ranged from 0.4 to 2.5 fmoles/ mg membrane protein. Human neoplasms originating in those tissues normally responsive to the trophic effects of gastrin have receptors for gastrin. Those tumors in which *G-17 binding was inhibited by secretin also possibly have a receptor for secretin that is distinct from the GR. (no Refs)

36. FREQUENCY OF 1,25-DIHYDROXYVITAMIN D3 RECEPTOR IN HUMAN BREAST CANCER.

Eisman JA, Suva LJ, Sher E, Pearce PJ, Funder JW, Martin TJ
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Cancer Res; 41(12):5121-5124 1981

Receptors for 1,25-dihydroxyvitamin D3 have been shown to exist in cultured breast cancer cells and in primary breast cancers. It is reported here that 1,25-dihydroxyvitamin D3 receptor (1,25-DR) was present in 80% of 54 unselected breast cancers. The concentration of 1,25-DR in the 43 receptor-positive tumors was 1.9 +-0.4 fmol/mg protein (SE). There was no correlation between 1,25-DR presence or concentration and the age of the patient or the concentration of estrogen, progesterone, androgen, or glucocorticoid receptors. 1-25-DR was also found in two of three renal cortical carcinomas but only in 3 of 14 gastrointestinal tract carcinomas. The relatively low concentration of 1,25-DR in these breast cancers, compared with that found in cultured breast cancer cells, is partially explained by incomplete 'exchange' with occupied receptors. Since the serum vitamin D-binding protein is not precipitated from serum itself or from tissue homogenates using the polyethylene glycol method, artifactual 1,25-DR levels due to the inevitable contamination of tissue specimens with this protein can be excluded. These findings indicate that 1,25-DR is not a nonspecific marker of cancer. The high frequency of 1,25-DR in the breast cancers may be related to the calcium-transport-ing ability of breast cancer cells which allows them to grow as osteolytic metastases. (Author abstract) (23 Refs)

37. EVIDENCE FOR A FOLIC ACID BINDING PROTEIN IN HUMAN CELL MEMBRANE.

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Acta Haematol (Basel); 66(3):202-209 1981

Cell membranes were prepared by sucrose discontinuous gradient from human liver and human peripheral WBC and RBC and from circulating WBC from patients with chronic granulocytic leukemia (CGL) and acute myeloblastic leukemia (AML). The membrane preparations from liver and from leukemic WBC were shown to bind tritiated folic acid. The membranes from normal WBC and RBC did not show this binding capacity. The membrane preparation from liver and CGL WBC showed two peaks of binding eluting with proteins from Sephadex G-200. However, protein extracts of these membrane preparations showed only a single peak for labelled folic acid, eluting near but just after albumin. The binding capacity of the membranes for folic acid was partially inhibited by reduced folate analogues. It is concluded that the liver plasma cell membrane and the membranes of myeloblasts in AML and circulating WBC in CGL contain a binding protein for folic acid which may be concerned in the transport of folates into these cells. (Author abstract) (22 Refs)

MEMBRANE/ SURFACE PROTEINS

38. INTERFERON INCREASES THE ABUNDANCE OF SUBMEMBRANOUS MICROFILAMENTS IN HELA-S3 CE LLS IN SUSPENSION CULTURE.

Wang E, Pfeffer LM, Tamm I
Rockefeller Univ., New York, NY, 10021
Proc Natl Acad Sci USA; 78(10):6281-6285 1981

Human beta(fibroblast) interferon inhibits the proliferation of human HeLa-S3 carcinoma cells in suspension culture. Accompanying this effect, the lateral mobility of cell surface receptors for concanavalin A is decreased and the rigidity of the plasma membrane lipid bilayer is increased. The present findings show a marked increase in the number of polymerized actin-containing microfilaments 3 days after treatment of HeLa-S3 cells with beta-interferon (640 units/ml). The cortical region of the treated enlarged cells contains a thick and dense meshwork of 40-70 A microfilaments. The actin nature of the filaments was verified by their ability to bind heavy meromyosin. These results support the concept that beta-interferon induces a coordinated response in the plasma membrane and the underlying microfilaments in both tumor and normal cells. (Author abstract) (35 Refs)

39. SUBCELLULAR LOCALIZATION AND ISOLATION OF GAMMA-GLUTAMYLTRANSFERASE FROM RAT HEPATOMA CELLS.

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Biochim Biophys Acta; 661(2):191-198 1981

Cultured rat hepatoma cells were homogenized and subjected to subcellular fractionation by analytical sucrose density centrifugation to determine the localization of gamma-glutamyltransferase ((5-glutamyl)-peptide:amino-acid 5-glutamyltransferase, EC 2.3.2.2). The activity was exclusively localized to the plasma membrane. Diazotized sulfanilic acid was used as a non-penetrant membrane reagent which inactivates ectoenzymes. With both intact and sonicated cells, only 70-75% inhibition of gamma-glutamyltransferase activity was observed. At least 12% of the total cell complement of gamma-glutamyltransferase activity is highly resistant to inactivation by diazotized sulfanilic acid even after Triton X-100 solubilization. The enzyme was purified from hepatoma cells and its properties compared with enzyme from normal liver. Apart from the striking increase in Vapp there were only minor differences between the enzymes from the two sources. In contrast to the complete abolition of transpeptidase activity of the purified hepatoma enzyme by diazotized sulfanilic acid, the hydrolytic activity of this preparation was only slightly inhibited. (Author abstract) (28 Refs)

40. MEMBRANE GLYCOPROTEINS INVOLVED IN CELL-SUBSTRATUM ADHESION.

Knudsen KA, Rao PE, Damsky CH, Buck CA
Wistar Inst. Anatomy and Biology, Philadelphia, PA, 19104
Proc Natl Acad Sci USA; 78(10):6071-6075 1981

A combination of immunological and biochemical methods were used to identify surface membrane components involved in cell-substratum adhesion. Broad-spectrum antiserum, prepared against surface membranes from hamster cells, induced reversible rounding and detachment of hamster fibroblasts from a substratum in vitro. This phenomenon was inhibited by Nonidet P-40 extracts of hamster cells. Therefore, an antibody neutralization assay was developed to detect the presence of antigen during the fractionation of Nonidet P-40 extracts of cells. After two differential precipitation steps, anion exchange chromatography, and sequential lectin affinity chromatography, a fraction greatly enriched in ability to block antiserum-induced changes in cell adhesion and appearance was isolated. Analysis of this fraction by NaDodSO4/polyacrylamide gel electrophoresis revealed a highly restricted group of glycoproteins with Mr = approx 140,000. A lectin-purified glycoprotein fraction was used to raise a higher titer antiserum that was able to induce reversible rounding and detachment of cells from a substratum and, when immobilized on an antibody affinity column, was able to bind and release material capable of blocking antiserum-induced cell rounding. These methods have allowed us to focus attention on a restricted group of glycoproteins that are integral constituents of the surface membrane and which play some as yet undetermined role in the process of cell-substratum adhesion. (Author abstract) (31 Refs)

41. MEMBRANE DIFFERENTIATION IN HUMAN MYELOID CELLS: EXPRESSION OF UNIQUE PROFILES OF CELL SURFACE GLYCOPROTEINS IN MYELOID LEUKEMIC CELL LINES BLOCKED AT DIFFERENT STAGES OF DIFFERENTIATION AND MATURATION.

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Proc Natl Acad Sci USA; 78(10):6299-6303 1981

Cell surface glycoprotein structures of human leukemic cell lines blocked at various stages of myeloid (granulocyte-monocyte) differentiation and maturation were characterized. Cell lines representing early stages of differentiation and maturation such as KG1a, KG1 and those representing later stages such as

ML-1-3 and HL-60 were found to express discretely different sets of cell surface glycoproteins. The proteins (such as Gp130) present on mature granulocytes appear only in cell lines representing later stages of maturation. The quantity and structure of the carbohydrate chains susceptible to endo-beta-galactosidase ('lactosaminoglycan') are also significantly different among cell lines. A small quantity of essentially unbranched lactosaminoglycan is present on KG1a and KG1 cells, whereas a significant quantity of branched lactosaminoglycan is present on the more mature ML-1-3 and HL-60 cells. The process of myeloid maturation from promyeloblast to granulocyte is accompanied by the disappearance of the and 95-kilodalton glycoproteins, the appearance of Gp130, and a, great increase in the quantity and branching str ucture of lactosaminoglycan. These results are discussed together with our previous findings on erythroid differentiation and maturation. (Author abstract) (36 Refs)

42. DIFFERENTIATING LEUKEMIA CELL LINES AS MODELS OF HEMATOPOIETIC MATURATION (MEETING ABSTRACT).

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Exp Hematol; 9(Suppl9):164 1981

We have been studying human leukemia cell lines endowed with the capacity of in vitro inducible functional and/or morphological differentiation. The cell lines have been chosen to represent main lineages of hematopoietic system. The K562 cell line was originally established from the pleural effusion of a patient with chronic myeloid leukemia in blast crisis. We found that this cell line expressed features of an early erythroblast, and could be introduced to enhanced Hb synthesis and morphological differentiation in vitro with sodium butyrate or diazepam. Remarkable changes in the surface glycoprotein profile and the intracellular distribution of spectrin were observed during the induced differentiation. The HL-60 promyelocytic leukemia line is inducible to terminal granulocytic differentiation in the presence of dimethyl sulfoxide. During this maturation the cells acquire phagocytic and chemotactic capacity. We found a change in the mobility of the main surface sialoglycoprotein of the cells during induced differentiation. The GP160 disappeared and a new band, GP130, was seen. GP130 might be involved in the chemotactic response since this protein was not found by carbohydrate labeling of the surface proteins or granulocytes of patients with monosomy 7, whose cells do not respond to chemotactic stimuli. We have recently established a continuous cell line, JOK-1, from the blood of a patient with hairy cell leukemia (HCL). This cell line showed the phenotype of an early B cell and it grows in suspension with a rather smooth outer surface. When cultivated in the presence of sodium butyrate and hairy morphology is acquired with surface expression of monoclonal kappa-light chains and increased tartrate resistant acid phosphatase activity. (3 Refs)

43. EXPRESSION OF DR ANTIGENS IN CHRONIC LYMPHOCYTIC LEUKEMIA (MEETING ABSTRACT).

Cassol S, Campbell L, Bryant L, Muzik H, Jerry LM
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Oncodev Biol Med; AbstractsBook,1:P19 1981

HLA-DR (or Ia-like) antigens detergent solubilized from cell membranes of leukemic blasts of patients with chronic lymphocytic leukemia of the B cell variety were purified by gel filtration followed by affinity chromatography on concanavalin A Sepharose. After radioiodination, the DR antigens were immunoprecipitated with specific antisera and analyzed by two-dimensional gel electrophoresis. Repe ated mapping revealed several different patterns of DR antigen expression. Most patients (18 out of 35) gave map patterns resembling those obtained from normal peripheral blood B cells. Such maps revealed light (27K) and heavy (33K) DR chains as well as a non-dissociated 60K DR complex. All components, and especially DR light chains, were polymorphic as shown by their further separation into several isoelectric variants. Maps from six patients showed decreased levels of 33K and 60K antigen components, while nine patients expressed only DR light chains. Two patients lacked detectable DR antigens. Moreover, sequential mapping of individual patients indicated that 33K and 60K DR components fluctuate in relation to disease activity. This temporal fluctuation was independent of other B cell markers including a newly defined surface membrane protein of 28,000 daltons. At present, leukemic patients are being classified according to map pattern in the hope that the loss of DR antigen expression in certain patients can be related to the state of differentiation and to the clinical aggressiveness of individual tumors. (no Refs)

44. HETEROGENEITY IN SURFACE GLYCOPROTEINS OF MOUSE PERITONEAL MACROPHAGE POPULATIONS.

Kaplan G, Olstad R
Inst. Medical Biology, Univ. Tromso, Tromso, Norway
Exp Cell Res; 135(2):379-386 1981

Murine peritoneal macrophages were activated in vitro by repeated addition to the culture medium of cell-free tumor ascites fluid from a syngeneic methylcholanthrene-induced sarcoma Cell morphology was studied by phase-contrast microscopy and scanning electron microscopy (SEM). The ascites-activated cells were extensively spread out on the glass surface and showed extensive membrane ruffling. To investigate the protein metabolism of the cells, total protein content and incorporation and turnover of [14C]glucosamine and [35S]methionine into TCA-precipitable macromolecules were analyzed. Activated cells had a total protein content 2-3x higher than that found in control cells. [35S]Methionine and [14C]glucosamine were incorporated into ascites-treated cells at a rate 2.7-3x higher than into control cells. Cell glycoproteins were lost with biphasic kinetics with a reduced half-life for both phases in the activated cells as compared to controls. Radiolabelled proteins were isolated from the plasma membrane by immunoprecipitation of trinitrobenzene sulfonic acid-derived cells and analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Whereas most of the protein peaks were the same in both cultures, the control cells had a single broad protein peak in the range of 100-110 K, whereas the ascites-activated cells had three peaks of 90, 100-110 and 135 K. The 135 K peak appeared to be a 'new' surface glycoprotein expressed in the activated but not in the control cells. (Author abstract) (29 Refs)

45. EVIDENCE FOR THE ASSOCIATION OF TWO CELL SURFACE GLYCOPROTEINS OF 13762 MAMMARY ASCITES TUMOR CELLS: CONCANAVALIN A-INDUCED REDISTRIBUTION OF PEANUT AGGLUTININ-BINDING PROTEINS.

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Exp Cell Res; 135(2):418-424 1981

The behavior of the cell surface concanavalin A (con A) receptors and of peanut agglutinin (PNA) receptors on the MAT-B1 ascites subline of the 13762 rat mammary adenocarcinoma was examined using fluorescein-labeled conA and PNA. ASGP-1, the major glucosamine-containing glycoprotein of these ascites cells, is the only PNA-binding protein observed by dodecyl sulfate electrophoresis. ASGP-2, the second most prominent component after glucosamine labeling, is the most abundant conA-binding protein. These two glycoproteins were previously shown to be associated as a complex in detergent extracts of the cells. ConA-binding proteins, upon incubation with fluorescein-labeled conA (FITC-conA), redistribute on the cell surface into small and large aggregates similar, but not identical, to those seen in 'patching' and 'capping' experiments with lymphocytes. PNA-binding proteins failed to redistribute during incubation with fluorescein-labeled PNA (FITC-PNA) and appeared in a diffusely stained pattern around the circumference of the cells. However, when cells were treated with unlabeled conA followed by FITC-PNA, or with FITC-PNA followed by unlabeled conA, there was marked redistribution of the FITC-PNA. These results indicate that ASGP-1 redistributes in response to the movement of conA-binding proteins and supports our hypothesis that ASGP-1 and ASGP-2 are associated on the plasma membrane at the cell surface as well as in detergent extracts. (Author abstract) (22 Refs)

46. THE DETECTION AND CHARACTERIZATION OF A MEMBRANE PROTEIN WITH FACTOR B-LIKE ACTIVITY ON HUMAN LYMPHOID CELLS.

Woo P, Lachmann PJ
Mechanisms in Tumor Immunity Unit, MRC Centre, Hills Road, Cambridge, England
Immunology; 44(3):629-639 1981

Factor B-like activity associated with human peripheral blood lymphocytes was first described by Halbwachs and Lachmann (1976), who employed a functional assay. In this present study, clones of B cells from patients with chronic lymphatic leukemia (CLL) and lymphoblastoid 'Raji' cells were used. The surface of these cells was labeled with 125I by the lactoperoxidase technique and the cell membrane proteins solubilized with the detergent NP40. A single polypeptide chain of mol wt 103K was precipitated with F(ab')2 anti-serum Factor B, and not with a control antibody. This 103K protein was also found if Raji cells were biosynthetically labeled with [14C]leucine. When the radiolabeled cells were incubated with cobra venom factor (CVF) and Factor D, two specific polypeptides were precipitated by F(ab')2 anti-Factor B. Moreover, the larger fragment forms a complex with CVF, since it could be precipitated by anti-CMF. These events are similar to those involving serum Factor B and CVF. (Author abstract) (32 Refs)

47. HUMORAL IMMUNE RESPONSE IN MELANOMA AND GLIOMA PATIENTS: A SOLUBILIZED MELANOMA-MEMBRANE COMPONENT AS A TOOL FOR DETECTING CIRCULATING ANTIBODIES.

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Cancer; 48(11):2399-2408 1981

In search of tumor-associated components in human melanomas and gliomas, plasma membranes were isolated from these tumors and compared morphologically, biochemically and immunologically with those from normal human brain. The preparations exhibited a comparable ultrastructure of empty vesicles of various form and size. The melanoma membrane consisted of at least 15 protein components and the glioma membrane consisted of at least 24 protein components, 4 of which were absent in the membrane of normal tissue, as revealed by polyacrylamide gel electrophoresis. The four tumor-associated polypeptides could be solubilized together with a few other membrane components by the non-ionic detergent Triton-X100 and were used as an antigen source for immunologic characterization of melanoma and glioma membranes. A rabbit antiserum prepared against these solubilized membrane components from melanoma and glioma membranes. A rabbit antiserum prepared against these solubilized membrane components from melanoma was absorbed with plasma membranes from a variety of normal tissue including RBC, lymphocytes, spleen, liver, lung, kidney, placenta and brain. The absorbed antiserum reacted with the membrane extract of all the 11 melanomas tested so far; in addition it crossreacted with an identically prepared extract from all the 15 gliomas tested. Membrane extracts from other tumors, such as kidney and mammary carcinoma, were negative in this respect. Furthermore, a rabbit antiserum prepared against the Triton extract from glioma membranes yielded after exhaustive absorption an immune precipitate not only with the specimens from the 15 other gliomas but also with those from the 11 melanomas. Electrophoretic analysis of the immune precipitate formed by the Ig fraction of the absorbed rabbit antiserum and the Triton-X100 extract from melanoma membranes exhibited six polypeptide bands, two of which appear antigen-derived as they were missing in the profile of the Ig pattern. A comparable electrophoretic pattern was obtained with the immune precipitate of the glioma antigen specimen. The mol wt of these two polypeptide was estimated on the basis of their electrophoretic mobility to be 55,000 and 10,000 daltons, respectively. The electrophoretic immunologic findings argue for a common membrane component in human melanoma and glioma patients. Using the Triton extract from melanoma membranes as an antigen source, precipitating antibodies could be detected in the Ig fraction of 16 of the 24 sera from melanoma patients and in that of 15 of the 23 glioma patients by means of counter-current electrophoresis. Sera from a variety of cancer patients with diseases other than melanoma or glioma as well as from patients without cancer were negative in this respect. (Author abstract) (37 Refs)

48. ASSOCIATION OF A 68,000-DALTON PROTEIN WITH ADRENOCORTICOTROPIN-SENSITIVE ADENYLATE CYCLASE ACTIVITY IN Y1 ADRENOCORTICAL TUMOR CELLS.

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J Biol Chem; 256(21):11365-11371 1981

This report explores the biochemical basis for clonal variation in adrenocorticotropin (ACTH)-sensitive adenylate cyclase activity in the Y1 mouse adrenocortical tumor cell line. We demonstrate that the level of a specific protein, designated p68, is significantly correlated with the ability of adrenocorticotropin to stimulate adenylate cyclase activity among Y1 subclones ($p = 0.004$; $r = 0.65$). p68 was characterized by its mol wt in sodium dodecyl sulfate polyacrylamide gels ($Mr = 68{,}000$) and by its isoelectric point as determined by two-dimensional gel electrophoresis ($pI = 7.2$). On two-dimensional gels, the protein migrated as a major spot with satellite spots 0.1 pH unit on either side. Homogenates and plasma membrane fractions from clones highly responsive to ACTH had large amounts of p68. In homogenates from highly responsive clones, p68 represented 10 to 12% of the total protein. Homogenates and plasma membrane fractions from clones insensitive to ACTH were deficient in p68. In homogenates from the insensitive clones Y6 and OS3, p68 represented less than or equal to 0.8% of the total protein. A somatic cell hybrid, formed by fusion of these two ACTH-insensitive clones recovered ACTH-sensitive adenylate cyclase activity and concomitantly expressed appreciable levels of p68. It is suggested that p68 may regulate the transfer of information from the occupied ACTH receptor to the catalytic subunit of adenylate cyclase. (Author abstract) (28 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

49. SYNTHESIS AND PROCESSING OF THE ALPHA HEAVY CHAINS OF SECRETED AND MEMBRANE-BOUND IGA.

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Proc Natl Acad Sci USA; 78(10):6436-6440 1981

We have compared the synthesis and processing of Ig alpha chains in two murine cell lines, a B cell lymphoma that expresses membrane-bound IgA and a hybridoma that secretes IgA. Results of biosynthetic labeling experiments demonstrated that membrane-bound and secreted alpha chains have two distinct intracellular precursors, of different mol wt and isoelectric points. RNAs from both of these cell lines direct the synthesis in vitro of two alpha polypeptides of Mr 59,000 and 62,000, the larger one being the precursor for membrane-bound alpha chain and the smaller one being the precursor for secreted alpha chain. These cell lines each contain three RNAs, 1.7, 2.1, and 3.1 kilobases in length, which hybridize with cDNA for the alpha constant region and which are present in different concentrations. Our results suggest that the smallest RNA encodes the secreted alpha chain and one or both of the larger RNAs encode(s) the membrane-bound alpha chain. (Author abstract) (33 Refs)

50. DEVELOPMENT OF THERMOTOLERANCE IN CULTURED REUBER H35 RAT HEPATOMA CELLS (MEETING ABSTRACT).

Schamhart DH, Van Rijn J, Van Walraven HS, Wiegant F, Van Wijk R
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Strahlentherapie; 157(9):622 1981

Exposure of mammalian cells to elevated temperatures (hyperthermia) has been reported to induce an increased ability of cells to survive thermal stress (thermotolerance). Furthermore, heat has been shown to act at the level of initiation of messenger-RNA translation. We considered it to be of interest to study protein synthesis in relation to the development of thermotolerance. Cultured Reuber H35 cells develop maximal thermotolerance 3 or 4 hr after either a 60-min treatment at 42.3 C followed by 37 C incubation or a continuous treatment at 41 C. However, whereas an initial treatment at 42.3 C results in 70% cell death, hardly any cell death is observed during continuous treatment at 41 C. In cells treated for 30 min at 42.3 C the rate of protein synthesis but thermal resistance of protein synthesis decreases to 15-30% of the control value. Subsequent incubation at 37 C results in a gradual resumption of protein synthesis. During the recovery a transient less inhibitory effect of hyperthermia on protein synthesis is observed, which becomes maximal 3 hr after the initial heat treatment. Continuous treatment at 41 C has no influence on the rate of protein synthesis develops. Thus, protein synthesis decreases to 20% of the control value if the cells are treated at 42.3 C shortly after the start of the 41 C incubation, while after longer incubation at 41 C (3 hr) treatment at 42.3 C has no effect. During the development of thermotolerance, specific proteins appear to be synthesized. An almost identical set of newly synthesized proteins can be detected under every condition studied. These results show that under certain hyperthermic conditons Reuber H35 cells develop thermotolerance faster than cell death occurs. The development of thermotolerance correlates with a reduced thermal sensitivity of protein synthesis and with induction of the synthesis of specific proteins. (no Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

51. INHIBITION OF RAT LIVER AMINOACYL-TRNA SYNTHETASES IN VITRO AFTER ACUTE AND CHRONIC AFLATOXIN B1 ADMINISTRATION IN VIVO.

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Chem Biol Interact; 37(1):233-244 1981

In a cell-free system the influence of acute aflatoxin B1 administration on the fractions of the hepatic microsomal translation complex of female rats was examined and compared with the influence of chronic aflatoxin B1 treatment. Polypeptide synthesis determined by [14C]leucine incorporation with a postmitochondrial supernatant was inhibited by 80% 24 hr after acute aflatoxin B1 administration whereas inhibition was only 17% in animals 30 wk after chronic treatment. After acute aflatoxin B1 administration inhibition of protein synthesis was by 67%, mainly on the polysomal level, whereas inhibition with the pH 5 enzyme was only by 22%. After chronic aflatoxin B1 administration inhibition was by 18%, mainly with the pH 5 enzyme and by only 6% on the polysomes. The inhibition by pH 5 enzyme was further investigated with regard to aminoacyl-tRNA synthetases, components of pH 5 enzyme. Leucine-specific aminoacyl-tRNA synthesis was inhibited by 20% 24 hr after acute and by 12% 30 wk after chronic aflatoxin B1 treatment. Aminoacyl-tRNA synthetase activities in a crude synthetase preparation tested with five amino acids were found to be noncompetitively inhibited. It is concluded that the inhibition of protein synthesis after acute and chronic aflatoxin B1 administration is in part due to decreased aminoacyl-tRNA levels caused by inhibited aminoacyl-tRNA synthetases. (Author abstract) (39 Refs)

52. INTERFERENCE OF GLUCOSAMINE AND OTHER AMINOSUGARS ON BLAST TRANSFORMATION.

Mazzuca A, Maldini M, Andollina A
Laboratorio Analisi Cliniche, Ospedale M. Malpighi, Bologna, Italy
Boll Soc Ital Biol Sper; 57(11):1274-1277 1981

The toxicity of glucosamine and other aminosugars on normal and neoplastic cells was studied in mitogen-induced lymphocytes. Incorporation of labeled leucine, uridine, and thymidine, precursors of protein, RNA and DNA, respectively, was strongly inhibited in blast cells exposed in vitro to D-glucosamine, D-galactosamine, and D-mannosamine. The possibility that glucosamine interfers with protein synthesis by preventing glucose metabolism was examined by adding increasing units of glucose or pyruvate to the culture medium. The results indicated that added glucose and pyruvate did not decrease the inhibitory effect of glucosamine. Glucosamine-6P, acetylglucosamine, and acetylgalactosamine produced only 20% inhibition when used at the same molar concentrations as glucosamine, galactosamine, and mannosamine. Since glucosamine-6P produced relatively minor metabolic interference in comparison with that of gluc osamine, it was suggested that glucosamine acts by interfering with the process of phosphorylation at various levels. It was suggested that the relative insensitivity of transforming lymphocytes to N-acetylglucosamine and N-acetylgalactosamine is based on their limited intracellular accumulation. (10 Refs)

53. DIFFERENCES IN OXYGEN-DEPENDENT REGULATION OF ENZYMES BETWEEN TUMOR AND NORMAL CELL SYSTEMS IN CULTURE.

Simon LM, Robin ED, Theodore J
Dept. Medicine, Stanford Univ. Sch. Medicine, Stanford, CA
J Cell Physiol; 108(3):393-400 1981

The effect of sustained O2 depletion on pyruvate kinase (PyKi), phosphofructokinase (PFK), cytochrome oxidase (CyOx), and superoxide dismutase (SOD) activities in cultured human fibrosarcoma cells (FS cells: HT 1080) and HeLa cells was measured and compared with the response of normal rat (RLF) and human (WI-38) lung fibroblast cell lines. In RLF and WI-38 cells, PyKi and PFK activites were increased following sustained hypoxia whether expressed per mg cell protein or per 10(6) cells. Activities (per mg protein) of CyOx and SOD were significantly lower in RLF following 96 hr of hypoxic cultivation as compared to values in air maintained cells. In contrast to fibroblast data, no significant differences in PyKi or PFK activities (whether expressed per mg protein or per 10(6) cells) were found between FS cells exposed to normal and low O2 tensions for 96 hr. As with FS cells, HeLa cells showed no significant effect of 96-hr exposure to low O2 tensions on either PyKi or PFK activities. Hypoxia (96-hr) did not significantly alter FS CyOx or SOD activities. Control values (T0) for PyKi and PFK per mg protein were significantly greater in FS cells than in RLF and WI-38 cells. Activities of CyOx, in contrast, were markedly decreased in FS cells at T0 as compared to the values in RLF cells. The results showed a striking difference in the response to sustained O2 depletion between specific and non-cancer cell lines during hypoxic cultivation. (29 Refs)

54. EFFECT OF HEAT SHOCK ON PROTEIN SYNTHESIS BY NORMAL AND MALIGNANT HUMAN LUNG CELLS IN TISSUE CULTURE.

Tsukeda H, Maekawa H, Izumi S, Nitta K
Dept. Virology, Natl. Inst. Health, Shinagawa-ku, Tokyo 141, Japan
Cancer Res; 41(12):5188-5192 1981

Heat shock proteins were found in cultured human lung cells by sodium dodecyl sulfate:gel electrophoresis and autoradiography using [35S]methionine. The synthesis of a mol wt 70,000 protein was markedly stimulated in normal, malignant, and SV40-transformed cells, and that of mol wt 90,000 and mol wt 100,000 proteins was stimulated only in malignant and SV40-transformed cells after heat shock treatment. In contrast, the synthesis of mol wt 200,000 and mol wt 250,000 proteins observed in the unheated normal cells was diminished after the same procedure. The heat shock proteins were induced within a given ra nge of temperature and duration of treatment, the conditions for normal and malignant cells being clearly different, ie, optima of 43 C for 1 hr in the normal cells and 41 C for 1 hr in the malignant cells. The results suggest that the analysis of heat shock protein is very useful for identifying the differential heat susceptibilities of normal and malignant cells and for elucidating their bases and mechanisms. (Author abstract) (27 Refs)

55. THE INDUCTION OF ORNITHINE DECARBOXYLASE BY TUMOR PROMOTING PHORBOL ESTER ANALOGUES IN REUBER H35 HEPATOMA CELLS.

Wu VS, Byus CV
Div. Biomedical Sciences, Univ. California, Riverside, CA, 92521
Life Sci; 29(18):1855-1863 1981

The induction of ornithine decarboxylase activity was studied in a rat hepatoma cell line (Reuber H35) incubated with a group of structurally-related phorbol ester analogues. A single application of 1.6 uM of tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) to H35 cells caused a dramatic increase in the activity of ornithine decarboxylase. The stimulation of the enzyme activity was rapid but transient, peaking at 4 to 5 hr with a value which was 116-fold greater than control and then declining to the basal level after 8 hr. In addition, the increase in ODC activity was dependent upon the concentration of TPA added to the culture medium and the EC50 was estimated to be about 2.63 x 10(-7) M. Our studies of the effect of various phorbol ester analogues on the H35 ODC activity indicated an apparent correlation between the ability of phorbol ester derivatives to induce ODC activity in the H35 cells and their activity to promote papilloma formation in the mouse skin in that the various derivatives possessed the following relative abilities to increase ODC activity: TPA greater than PDB greater than PDA greater than 4 alpha-P greater than 4 alpha-PDD. Concurrent addition of either actinomycin D or cycloheximide abolished the increase in ODC activity after TPA treatment. Changes of intracellular concentrations of polyamines, particularly putrescine, were in good agreement with the increase in ODC activity in response to TPA: a 10-fold increase in putrescine over the control level was observed at 6 hr. Our data suggest that cultured Reuber H35 hepatoma cells exhibit a marked and specific response to the phorbol ester tumor promoters and may be of great value in studying the biochemical mechanism of ODC induction by these agents. (Author abstract) (33 Refs)

56. SOME EFFECTS OF TREATMENT OF HEN ERYTHROCYTES IN VITRO WITH N-METHYL-N-NITROSOUREA.

Ward TH, Itzhaki RF
Paterson Lab., Christie Hosp., Manchester M20 9BX, England
J Cell Sci; 51:153-162 1981

Studies have been made of the effect of N-methyl-N-nitrosourea on hen RBC in vitro. These were done to find whether the highly condensed state of the chromatin and the very low metabolic activity of these cells would affect the extent of methylation of the DNA and chromatin proteins and the persistence of any methylation sites in these macromolecules with time after treatment. Also, the effect of methylnitrosourea on incorporation of [3H]uridine into RNA has been examined. It has been found that the DNA, histones and non-histone proteins are methylated. The main methylation site in DNA is 7-methylguanine and its level is higher that that found by others in the DNA of other cell types after treatment with methylnitrosourea; however, methylation of the two types of protein (especially the histones) is relatively very low. The level of methylation decreases in the DNA and the chromatin proteins with time after treatment. The amount of [3H]uridine in RNA was found to decrease after the treatment. (Author abstract) (19 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

57. INDUCTION OF ORNITHINE DECARBOXYLASE IN CULTURED MAMMARY TUMOR CELLS (MEETING ABSTRACT).

Cohen LA, Van der Snoek L
Naylor Dana Institute for Disease Prevention, Valhalla, NY, 10595
In Vitro; 17(3):242 1981

At present, little is known concerning the role played by polyamines in mammary cancer. Since in vivo studies are difficult to interpret at the cellular level, we have developed a defined in vitro system consisting of neoplastic epithelial cells derived from an N-nitrosomethylurea induced rat mammary adenocarcinoma. Ornithine decarboxylase (ODC), the rate-limiting step in polyamine synthesis, was measured in these cells by the in situ method of Patterson and Maxwell. To induce ODC, late log-phase cells were grown in serum-depleted medium. The kinetics of ODC induction was similar to that reported in other cell lines. Specific activity (sp act) at 0, 15 and 60 min was less than 0.5, 3.25 and 6.30 nmoles carbon dioxide/mg prot/hr, respectively; between 1 and 2 hr little change in sp act was observed. The specificity of the reaction was assessed by addition of inhibitors of the reaction mixture for the 2 hr assay period. There was an absolute requirement for pyridoxal phosphate; the reaction product, putrescine, inhibited ODC in a logarithmic fashion, and the competitive inhibitor alpha-methylornithine reduced enzyme activity by half. (1 Ref)

58. DIFFERENCES IN POLYAMINE METABOLISM OF THE UNDIFFERENTIATED AND DIFFERENTIATED NEUROBLASTOMA CELLS.

Chen KY, Liu AYC
Dept. Chemistry, Rutgers Univ., New Brunswick, NJ, 08903
FEBS Lett; 134(1/2):71-74 1981

The metabolism of [14C]putrescine in undifferentiated and differentiated mouse neuroblastoma cells was compared. Results indicate a 50-60% decrease of labeling of the 18,000-Mr protein of differentiated neuroblastoma cells as compared to that of undifferentiated neuroblastoma cells. An 8-10-fold increase in the conversion of [14C] putrescine into amino [14C] acids via [14C] gamma-aminobutyric acid is also reported. (20 Refs)

OTHER RELATED STUDIES

59. EVIDENCE OF FAR ULTRAVIOLET LIGHT-MEDIATED CHANGES IN PLASMA MEMBRANE STRUCTURE AND FUNCTION.

Braun AG, Buckner CA
Dept. Radiation Therapy, Harvard Medical Sch., 50 Binney Street, Boston, MA, 02115
Biochim Biophys Acta; 648(2):263-266 1981

Irradiation of plasma membrane preparations with 254 nm light increases its apparent microviscosity as measured with fluorescent polarimetry. Doses of 3.10(4) J/m2 increase the fluorescent polarization of a diphenylhexatriene probe by 10%. A similar increase is seen when whole cells are irradiated. The fate of membrane protein following irradiation was examined using SDS-polyacrylamide gel electrophoresis. Increasing the 254 nm doses reduces the amount of material in distinct bands on the gel and increases the amount of very low mobility material. No new bands of Coomassie blue staining material were observed. Irradiation of whole cells inhibited their attachment to concanavalin A-coated surfaces, an indication of a change in membrane function. (Author abstract) (12 Refs)

60. REGULATION OF GENE EXPRESSION BY TUMOR PROMOTERS. III. COMPLEMENTATION OF THE DEVELOPMENTAL PROGRAM IN MYELOID LEUKEMIC CELLS BY REGULATING MRNA PRODUCTION AND MRNA TRANSLATION.

Hoffman-Liebermann B, Liebermann D, Sachs L
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Int J Cancer; 28(5):615-620 1981

Regulation of gene expression has been analyzed in different clones of mouse myeloid leukemic cells treated with the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA), the macrophage- and granulocyte-inducing protein MGI, and combined treatment with TPA and MGI. Two-dimensional gel electrophoresis was used to measure changes in the rate of synthesis of specific proteins and in the amount of corresponding translatable mRNAs assayed in the reticulocyte cell-free translation system. TPA induced different subsets of differentiation-associated protein changes in the different clones and the degree of response to TPA was not necessarily related to the degree of response to MGI. It is shown that TPA can induce protein changes either by inducing the synthesis of new mRNA, by increasing or decreasing the amount of pre-existing mRNA, or by modulating the translation of a constant amount of mRNA. Combined treatment with TPA and MGI resulted in an enhancement of protein changes induced by MGI or TPA alone and induced differentiation-associated protein changes not induced by either compound separately in differentiation-defective clones. This complementation of gene expression appeared to be due to each compound inducing functions not induced by the other, so that the combined treatment resulted in new gene expression. Complementation also occurred at the level both of mRNA production and of mRNA translation. It is suggested that the ability of TPA to regulate gene expression at the level of mRNA production and mRNA translation and to complement changes in gene expression induced by other compounds such as MGI are important functions for its role as a tumor promoter. (Author abstract) (34 Refs)

61. QUANTITATIVE MICROSPECTROMETRICAL DETERMINATION OF TOTAL PROTEIN AND TOTAL PROTEIN-SH CONTENT OF THE NUCLEI OF EHRLICH ASCITES TUMOR CELLS WITHOUT PRIOR ISOLATION.

Nohammer G, Khoschsorur G
Halbarthgasse 5/I, Institut fur Biochemie, Universitat Graz, A-8010 Graz, Austria
Microsc Acta; 85(1):19-24 1981

Total protein and total protein-SH (thiol) content of both the cytoplasmic fraction and nuclei of Ehrlich ascites tumor cells as determined by macroscopic and microspectrophotometric methods showed fairly good agreement. Values of total protein and protein thiols for both whole cell and isolated nuclei differed by approx 20% with the two methods, while values for protein thiols for whole cells gave nearly identical values. (7 Refs)

62. CHEMOTACTIC PEPTIDE STIMULATED ENDOGENOUS ARACHIDONIC ACID METABOLISM IN HL60 GRANULOCYTES.

Bonser RW, Siegel MI, McConnell RT, Cuatrecasas P
Molecular Biology Dept., Wellcome Res. Lab., Research Triangle Park, NC, 27709
Biochem Biophys Res Commun; 102(4):1269-1275 1981

The chemotactic peptide N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys was found to activate endogenous arachidonic acid (AA) metabolism in granulocytes of the human promyelocytic leukemia cell line HL60. The N-formylated hexapeptide stimulated the rapid, dose-dependent release of free AA in dimethylsulfoxide-differentiated HL60 cells. Free AA reached a peak within 1 min after addition of the peptide. During chemotactic peptide stimulation there also was rapid synthesis of the lipoxygenase products 5-S-hydroxy-6,8,11,14-icosatetraenoic acid and leukotriene B4, which both reached peaks within 2 min. The cyclooxygenase product 12-L-hydroxy-5,8,10-heptadecatrienoic acid was also generated. AA was found to be incorporated into triacylglycerols and phosphatidylinositol. Stimulation of endogenous AA metabolism by N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys was blocked by the competitive antagonist Boc-Phe-Leu-Phe-Leu-Phe. The results indicated that the chemotactic peptide activates endogenous AA metabolism by a specific receptor-mediated event. (27 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Albrechtsen R 24
Altmannsberger 13
Andersson LC 42
Andollina A 52
Ashwell G 31
Avrameas S 23
Bagli NP 28
Bambara LM 37
Barnes DW 21
Bechtel PJ 12
Birkmayer GD 47
Blaser K 25
Bloom SR 30
Bluthmann K 3
Bonser RW 62
Braun AG 59
Brink L 17
Bryant L 43
Buck CA 40
Buckner CA 59
Bumol TF 29
Busch H 6
Busch H 9
Busch RK 9
Byus CV 55
Campbell L 43
Carraway KL 45
Cassol S 43
Char DH 33
Chauzy C 19
Chawla RK 18
Chen KY 58
Christman J K 7
Chung AE 27
Cohen LA 57
Cole RD 1
Corrocher R 37
Cotner T 25
Creusot F 7
Cuatrecasas P 62
Damsky CH 40
Darmon M 21
De Sandre G 37
DeMartino S 22
Del Valle U 17
Delpech B 19
Dighiero G 23
Ding JL 39
Doffoel M 11
Duceman BW 8
Eisen HN 25
Eisman JA 36
Engvall E 24
Eppenberger U 34
Erikson RL 10
Flickinger RA 4
Foidart JM 20
Fukuda M 41
Funder JW 36
Gallo RC 2
Galloway DR 29
Galluser M 11
Gerber L 17
Girard N 19
Glabe CG 32
Good RA 49
Grabel LB 32
Grenier JF 11
Guetzow K 6
Guilbert B 23
Handloser K 34
Handschin J 34
Helm RM 45
Hoffman-Lieberm 60
Holscher A 13
Holtlund J 15
Illmensee K 3
Itzhaki RF 56
Izumi S 54
Jacob ST 8
Jerry LM 43
Kaplan G 44
Khoschsorur G 61
Kikutani H 49
Knudsen KA 40
Koeffler HP 41
Kriedberg J 25
Kristensen T 15
Kundahl E 4
Kung W 34
Lachmann PJ 46
Lai CY 17
Laland SG 15
Launay J-F 11
Lawson DH 18
Levy WP 17
Liebermann D 60
Liotta LA 20
Lischwe MA 9
Liu AYC 58
Long RG 30
MacManus JP 16
Maekawa H 54
Maldini M 52
Marescaux J 11
Martin GR 32
Martin TJ 36
Matsukage A 5
Mazzuca A 52
McConnell RT 62
Miller FW 18
Minowada J 41
Morgan AC 29
Moschera J 17
Mosher DF 27
Mundy GR 22
Murphy-Ullrich 27
Muzik H 43
Nakanishi Y 14
Natori S 14
Nielsen M 24
Nishizawa M 5
Nitta K 54
Nixon DW 18
Nohammer G 61
Oberley TD 27
Olivier A 19
Olson MO 6
Olstad R 44
Orly J 21
Osborn M 13
Ostvold AC 15
Pachor ML 37
Pantazis P 2
Pastan IH 12
Pearce PJ 36
Pehrson JR 1
Pestka S 17
Peters TJ 39
Pfeffer LM 38
Potter M 25
Pritchard J 30
Rae-Venter B 35
Rand N 33
Rao PE 40
Raul F 11
Reisfeld RA 29
Richards RL 9
Richman R 4
Richmond A 18
Robin ED 53
Rose KM 8
Rosen SD 32
Rowe DW 22
Rubinstein M 17
Rudman D 18
Ruoslahti E 24
Rutherford AV 12
Sachs L 60
Salomon DS 20
Sarin PS 2
Sarkar S 28
Schamhart DH 50
Schauer A 13
Schimmer BP 48
Sekimizu K 14
Seyedin SM 1
Shah PN 28
Shealy DJ 10
Sher E 36
Shively J 17
Siegel MI 62
Simon LM 53
Simon PM 35
Singer MS 32
Sitia R 49
Smith GD 39
Stavnezer J 49
Steer CJ 31
Stein PC 33
Stein S 17
Suva LJ 36
Taguchi YN 5
Takahashi T 5
Takahashi T 5
Tamm I 38
Tamura H 14
Tanabe K 5

Theodore J 53
Thompson JC 35
Tiedemann K 30
Townsend CM 35
Tsukeda H 54
Unterreiner AM 51
Van Rijn J 50
Van Walraven HS 50
Van Wijk R 50
Van der Snoek L 57
Wagner G 51
Walker LE 29
Walker RA 26
Wang E 38
Ward TH 56
Watt VM 48
Weber K 13
Wewer U 24
Whitfield JF 16
Wiegant F 50
Willingham MC 12
Woo P 46
Wu VS 55
Yaar M 20
Yamada SS 12
Yamaguchi M 5

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In this issue:

- **NUCLEOPROTEINS** 1-3
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** 4-6
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** 7-17
- **BINDING/ RECEPTOR PROTEINS** 18
- **MEMBRANE/ SURFACE PROTEINS** 19-20
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** 21-23
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** 24-26
- **DIETARY AND NUTRITIONAL EFFECTS** 27-28
- **POLYAMINES - SYNTHESIS AND METABOLISM** 29-30
- **OTHER RELATED STUDIES** 31-36

NUCLEOPROTEINS

1. REPAIR OF O(6)-ETHYLGUANINE IN DNA BY A CHROMATIN FRACTION FROM RAT LIVER: TRANSFER OF THE ETHYL GROUP TO AN ACCEPTOR PROTEIN.

Mehta JR, Ludlum DB, Renard A, Verly WG
Div. Oncology, Dept. Medicine, Albany Medical Coll., Albany, NY, 12208
Proc Natl Acad Sci USA; 78(11,part2):6766-6770 1981

Incubation of O6-[3H]ethylguanine-containing DNA with a rat liver chromatin fraction resulted in a decrease in the O6-ethylguanine content of the DNA. Analysis of the products of

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this reaction showed that the ethyl group had been transferred from the O6-ethylguanine to a protein acceptor. When the incubation mixture was separated on a cesium chloride gradient, the radioactivity removed from O6-ethylguanine appeared in a low-density band. This material has been isolated and subjected to trypsin digestion and high-pressure liquid chromatography analysis; it was sensitive to trypsin and the digest contained new high-pressure liquid chromatography peaks characteristic of oligopeptides. Radioactive peaks from the trypsin digestion have been digested further to the amino acid level and have been shown to contain S-[3H]ethylcysteine. Thus, we conclude that the repair activity in rat liver chromatin removes the ethyl group from O6-ethylguanine and transfers it to a cysteine moiety contained in an acceptor protein. (Author abstract) (23 Refs)

2. NUCLEAR PROTEIN KINASES OF GH3 CELLS.

Fabbro D, Jungmann RA, Eppenberger U
Hormone Lab. and Experimental Endocrinology, Univ. Clinic Medical Sch., CH-4031 Basel, Switzerland
Horm Res; 14(4):260-276 1981

Three distinct cAMP-independent nuclear protein kinase activities (PCv, PC-II and PC-III) have been separated by phosphocellulose chromatography of GH3 nuclear NaCl extracts. The kinase activities exhibit distinct substrate specificities and preference for either the basic substrates protamine sulfate and histones H1 and H3 (kinase activity PCv) or the acidic protein substrates casein (PC-II) and phosvitin (PC-III). The three nuclear protein kinases further differ in their estimated mol wt, apparent Km values and in their sensitivity toward NaCl. All three kinases require $Mg++$ for optimal activity. (Author abstract) (40 Refs)

3. EFFECT OF EMBRYONAL HISTONES ON MORPHOLOGICAL CHANGES AND SUPRAVITAL STAINING OF CULTURED TUMOR CELLS.

Krivchik AA, Ershova-Pavlova AA, Berlov GA, Dad'kov IG
State Medical Inst., Minsk, USSR
Dokl Akad Nauk BSSR; 25(10):952-955 1981

The effect of embryonal histones (EH) isolated from chick embryo and calf thymus was studied in cultured cells of human angiosarcoma, human stomach carcinoma, and human embryo tissue. Tumor cells incubated with EH showed marked swelling of cytoplasm and nucleus; cells contained hematoxylin-fast cytoplasmatic bodies. The most marked changes in the tumor cells were observed after incubation with arginine-rich EH; lysine-rich EH were the least effective. (11 Refs) (In RUS)

CYTOPLASMIC AND CONTRACTILE PROTEINS

4. EXPRESSION OF GLIAL AND VIMENTIN TYPE INTERMEDIATE FILAMENTS IN CULTURES DERIVED FROM HUMAN GLIAL MATERIAL.

Osborn M, Ludwig-Festl M, Weber K, Bignami A, Dahl D, Bayreuther K
Max Planck Inst. Biophysical Chemistry, D-3400 Goettingen, W. Germany
Differentiation; 19(3):161-167 1981

Several cultures established from biopsies of apparently normal adult human glial material showed no cells positive for glial fibrillary acidic protein (GFA) when examined after seven or more cumulative population doublings (CPD), although the established glioma line U251 MG showed approx 3% GFA-positive cells, and U333 CG/343 MG clone 3 showed greater than 98% GFA-positive cells. Both the human glia derived cultures and the glioma lines were positive when assayed with sera specific for vimentin. We therefore investigated the expression of GFA as a function of cumulative population doublings after the establishment of primary cultures. Under our experimental conditions, although GFA-positive cells were clearly present in the primary cultures accounting for some 3%-14% of the cells present, the GFA marker was subsequently lost, and the proliferating cultures expressed only the vimentin type of intermediate filament. Those cells that were GFA-positive also appeared to be vimentin-positive. GFA expression was not reinduced in cultures that had lost the GFA marker by treatment with dibutyryl cyclic AMP. We discuss two alternate hypotheses for the origin of the GFA-negative cells: (1) the cultures are of astrocyte origin but lose the ability to express GFA on culturing; (2) the cultures originate from cells of nonastrocyte origin present in the primary material. (Author abstract) (36 Refs)

5. TWO-DIMENSIONAL PROTEIN ELECTROPHORESIS AND THE IDENTIFICATION OF HISTOLOGICALLY INDETERMINATE HUMAN CANCERS.

Anderson KM, Baranowski J, Economou SG
Oncology Lab., Section Medical Oncology, Dept. Medicine, Rush Medical Coll., Chicago, IL, 60612
Med Hypotheses; 7(10):1303-1316 1981

Use of two-dimensional protein electrophoresis (PE) to identify histologically indeterminate cancers is presented. Such cancers account for 5-10% of a typical sample of cancer patients. Proteins from tumor cells were separated according to mol wt and isoelectric point. Comparison of protein profiles of adenoma of the prostate (AP) and adenocarcinoma of the lung (ACL) indicated that 96 of 153 proteins from AP were absent from ACL, and that 55 of 112 proteins from ACL were absent from AP. Identification of cancers by PE is based on the hypothesis that protein profiles of cancer cells are not random but reflect both the stem cells from which the cancer is derived and the stage at which cellular maturation stopped. Proteins analyzed include acidic and neutral proteins. Use of Coomassie stain allows detection of less than 1 ug of protein. If staining is inadequate, autoradiographic methods may be used. Manual analysis by comparison of profiles rather than computer analysis should suffice in most cases. Sample profiles are compared with profiles of known cancer types. (47 Refs)

6. TRANSFERRIN IN THE RAT PROSTATE DUNNING TUMOR.

Wilson EM, French FS, Petrusz P
Dept. Pediatrics, Univ. North Carolina, Chapel Hill, NC, 27514
Cancer Res; 42(1):243-251 1982

A major protein of the rat Dunning prostate tumor has been purified. It has physicochemical properties and an amino acid composition similar to that of transferrin. Furthermore, the isolated tumor protein reacts with antiserum to authentic rat transferrin. Immunoperoxidase staining with rabbit anti-rat transferrin localizes transferrin within tumor acinar glands. Rocket immunoelectrophoresis indicates that transferrin constitutes 30 to 40% of tumor fluid protein, but accounts for only approx 9% of total serum protein. In normal rat prostate cytosols, the level of transferrin is at least 200x lower than in tumor cytosol. Nevertheless, dorsal and lateral prostate show variable peroxidase staining indicating the presence of immunoreactive transferrin within acinar glands of these normal tissues. While intense staining for transferrin was found in the interstitium of all regions of the normal prostate, transferrin was not detected within acinar glands of the coagulating gland, ventral prostate, or seminal vesicle. Immunocytochemical localization of albumin indicates a distribution similar to that of transferrin in normal and neoplastic rat prostates. However, unlike transferrin, the albumin content was lower in tumor fluid than in serum. It is suggested that the high level of transferrin in tumor fluid may be due to selective uptake of serum by the tumor. (Author abstract) (36 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

7. RELATIONSHIP BETWEEN SERUM VISCOSITY AND INTRAVASCULAR IGA POLYMER CONCENTRATION IN IGA MYELOMA.

Chandy KG, Stockley RA, Leonard RC, Crockson RA, Burnett D, Maclennan IC

Immunodiagnostic Res. Lab., Univ. Birmingham Medical Sch., Birmingham B15 2TJ, England

Clin Exp Immunol; 46(3):653-661 1981

Thin-layer chromatography followed by crossed immunoelectrophoresis was used to examine the relationship between serum viscosity and IgA polymer concentration in 33/179 IgA myeloma patients. The range of relative serum viscosity (RSV) for serum from healthy donors was 1.25-1.7. An RSV of greater than 1.7 was observed in 149/179 of the myeloma patients. The RSV of the 33 patients examined further ranged from 1.31-5.88 with two patients having RSV of 9.51 and 9.76. The proportion of IgA polymer in the 33 patients ranged from 0-88%. The correlations between RSV and serum monomer equivalent IgA concentration (rs = 0.3091, p greater than 0.05), and RSV and actual IgA monomer concentration (rs = 0.0024, p greater than 0.1) were poor. A strong correlation was observed between RSV and the serum IgA polymer equivalent concentration (rs = 0.7928, p less than 0.001). On serial dilution of sera from 4/31 patients without marked hyperviscosity, the relative serum viscosity was observed to decrease rapidly at first and then more gradually. The two hyperviscous sera exhibited a more marked initial reduction in RSV. The RSV was higher at 23 C than at 37 C in the sera of 7/31 patients without marked hyperviscosity. The same temperature change resulted in a far greater increase in RSV in the two markedly hyperviscous sera. IgA polymers, larger than dimers, were demonstrated in 7/33 sera; none were observed in the sera of two patients with markedly hyperviscous sera. The serum hyperviscosity did not seem to be dependent on the presence if IgA polymers larger than dimers. IgA complexes with albumin, beta-1-lipoprotein, and alpha-1-antitrypsin were observed in some sera with lower RSV. However, IgA complexes with these plasma proteins were not detected in the two markedly hyperviscous sera. Protein aggregation seemed to be a major determinant in the development of serum hyperviscosity in the two patients with marked hyperviscosity. (22 Refs)

8. BOMBESIN, SOMATOSTATIN AND NEUROTENSIN-LIKE IMMUNOREACTIVITY IN BRONCHIAL CARCINOMA.

Wood SM, Wood JR, Ghatei MA, Lee YC, O'Shaugnessy D, Bloom SR

Dept. Medicine, Hammersmith Hosp., Du Cane Road, London W12 OHS, England

J Clin Endocrinol Metab; 53(6):1310-1312 1981

Bombesin, a regulatory peptide found in adult lung and in higher concentration during development in the fetal lung, has potent biological actions which include the ability to stimulate the release of a number of hormones. Radioimmunoassay of extracts of tumor, lung and plasma from patients with bronchial carcinoma revealed very high tissue bombesin concentrations in two oat cell carcinomas, with lower levels in two out of six adenocarcinomas exceeding the concentrations found in normal lung. The oat cell carcinomas also contained significant amounts of somatostatin and in one case neurotensin. Neurotensin was further detected in two out of seven squamous tumors and three adenocarcinomas. Secretion of regulatory peptides by bronchial carcinomas may account for some of their ill-understood non-metastatic clinical manifestations. (Author abstract) (17 Refs)

9. BIOCHEMICAL PROPERTIES OF DIFFERENTIATION FACTORS FOR MURINE MYELOMONOCYTIC LEUKEMIC CELLS IN ORGAN CONDITIONED MEDIA - SEPARATION FROM COLONY-STIMULATING FACTORS.

Nicola NA, Metcalf D

Cancer Res. Unit, Walter and Eliza Hall Inst. Medical Res., P.O. Royal Melbourne Hosp., Parkville, Victoria 3050, Australia

J Cell Physiol; 109(2):253-264 1981

Various murine (C57BL/6f/J/WEHI) organs were surveyed for their capacity to synthesize and release differentiation factor (DF) into the medium. The effect of prior injection of endotoxin (5 ug) on DF production was studied and the biochemical properties of DF produced under various conditions were compared with each other and with the DF found in the serum. Optimal strategy for purifying DF was described, with particular attention given to its separation from the proliferative stimulus colony-stimulating factor (CSF). All organs could induce differentiation of the myelomonocytic leukemic cell lines WEHI-3B, but were much more effective from endotoxin-injected animals. Production of DF was dependent on protein synthesis and occurred throughout the 7-day incubation period. DF produced by the different organs in vitro and found in vivo in endotoxin serum could be distinguished from the majority of granulocyte-macrophage (GM) and macrophage (M) CSF. DF was precipitated by 55% ammonium sulfate and did not bind to Concanavalin A-Sepharose; CSF required 85% saturation and bound to Concanavalin A-Sepharose. DF eluted later than CSF from Phenyl-Sepharose columns. DF could be distinguished from M-CSF by its apparent mol wt on native gels, but could not be distinguished from GM-CSF (23,000 on that basis). DF appears to be a 23,000 (approx) mol wt protein which is different from GM-CSF and may or may not be a glycoprotein. (26 Refs)

10. PURIFICATION AND CHARACTERIZATION OF THE PLASMINOGEN ACTIVATOR SECRETED BY A RAT BRAIN TUMOR CELL LINE IN CULTURE.

Bykowska K, Rijken DC, Collen D
(c/o Collen) Center for Thrombosis and Vascular Res., Dept. Medical Res., Univ. Leuven, 3000 Leuven, Belgium
Thromb Haemost; 46(3):642-644 1981

Purification and characterization of a plasminogen activator (PA) secreted in vitro by a rat brain tumor cell line is reported. Cells from line RT4-71-1 were grown to confluency. Serum-free medium with a plasminogen-dependent plasminogen activator activity of 9 IU/ml was obtained. PA was purified by chromatography on zinc-chelate-agarose, Concanavalin A, and Sephadex G-150 in the presence of 0.01% (vol/vol) Tween 80. Final yield was 39%. Electrophoresis produced one main band possessing PA activity with a mol wt of 60,000 and a minor band with a mol wt of 30,000. Amino acid (AA) analysis showed that rat brain tumor (RBT) PA and human tissue (HT) PA, obtained from a human melanoma line, had comparable AA composition. RBTPA and HTPA bound to fibrin clots, but urokinase (UK) did not. Antisera against HTPA quenched activity by HTPA and RBTPA but not by UK. Antisera against UK quenched activity by UK but not by HTPA or RBTPA. RBTPA evidently belongs to the class of PA not related to UK. (14 Refs)

11. BIOSYNTHESIZED PRODUCTS OF CULTURED NEUROGLIAL CELLS: I SELECTIVE RELEASE OF PROTEINS BY CELLS FROM HUMAN ASTROCYTOMAS.

McKeever PE, Quindlen E, Banks MA, Williams U, Kornblith PL, Laverson S, Greenwood MA, Smith B
Surgical Neurology Branch, Natl. Inst. Neurological and Communicative Disorders and Stroke, NIH, Building 10A, Room 3E68, Bethesda, MD, 20205
Neurology (NY); 31(11):1445-1452 1981

The proteins synthesized and released by human astrocytoma cells cultured with radiolabeled amino acids were analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate and by trichloroacetic acid precipitation. A select group of extracellular proteins was released from the astrocytomas. The most abundant extracellular proteins were at least 250,000 daltons. Five other major proteins (P 175, P 125, P 60, P 47, and P40) were 175,000, 125,000, 60,000, 47,000, and 40,000 daltons, respectively. The complement of proteins retained by the cells was considerably more complex than those released. Clinical efforts to enhance the immunologic response directed against human astrocytomas must distinguish between extracellular and retained antigens. The pattern of proteins released by gliomas might be diagnostically useful if present in cerebrospinal fluid or serum. (Author abstract) (48 Refs)

12. PURIFICATION AND CHARACTERIZATION OF AN IMMUNOSUPPRESSIVE FACTOR FROM OVARIAN CANCER ASCITES FLUID.

Oh SK, Moolten FL
Dept. Microbiology, Boston Univ. Sch. Medicine, 80 East Concord St., Boston, MA, 02118
Eur J Immunol; 11(10):780-788 1981

A nonspecific immunosuppressive factor present in malignant (ovarian carcinoma) ascites fluid has been purified by acid extraction from a high mol wt (greater than 200,000) complex followed by preparative isoelectric focusing on Bio-lyte media. It is an acidic protein (pI = 3.6) of mol wt 50,000 to 52,000 as estimated by gel filtration and composed of subunits of 25,000 to 26,000 estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, under reducing conditions. It inhibits the phytohemagglutinin-dependent mitogenic response of normal peripheral blood lymphocytes by 50% at 2 ug/ml concentrations in vitro and suppresses 80% of the plaque-forming cell response to sheep RBC at 100 ug per mouse in vivo. Its chemical identity with any of the known plasma proteins has not been established. Its failure to stain with periodic acid Schiff's reagent indicates its minimal content of carbohydrates. It is susceptible to tryptic and pronase digestion but insensitive to deoxyribonuclease and ribonuclease digestion. A smaller suppressive factor identified in the same fluid appears to be a lymphotoxin; it differs from the acid-extracted nonspecific suppressive factor in its lack of susceptibility to trypsin. (Author abstract) (31 Refs)

13. PLASMINOGEN ACTIVATOR ACTIVITY AND COMPOSITION IN HUMAN BREAST CANCER.

Evers JL, Patel J, Madeja JM, Schneider SL, Hobika GH, Camiolo SM, Markus G
(c/o Markus) Dept. Experimental Biology, Roswell Park Memorial Inst., New York State Dept. Health, Buffalo, NY, 14263
Cancer Res; 42(1):219-226 1982

The plasminogen activator activity of human breast tumors was determined quantitatively in extracts of 33 primary tumors, 11 lymph node metastases, and 11 normal tissues. Activities varied more widely in the neoplastic tissues than in the normal ones. The mean activator content of the tumors (primary and metastasis) was significantly higher (p greater than 0.01) than that of normal tissues: 4.4-fold on a g wet tissue basis and 2.1-fold when the activities were referred to the protein content of the extracts. Primary and metastatic tumors were not significantly different from one another in their plasminogen activator content. In the six cases where tumors and normal tissue pairs from the same patients could be compared, the mean of the individual tumor:normal ratios was 10.0 (wet wt) or 3.6 (extract protein). In addition to the activator content, the molecular type of the enzymes was also determined, using goat antibody formed against human urinary urokinase as an inhibitor of activator activity. On the av, 80% of the activity of the tumor extracts could be inhibited by the antibody; extracts of normal breast tissue contained only 62% urokinase-like activator. The activators were also characterized by sodium dodecyl sulfate gel electrophoresis using two different methods for the zymographic visualization of activator bands. The fraction of urokinase-like enzyme determined by the fibrinogen copolymerization method agreed well with the results obtained by immunoinhibition. No correlation was found between activator activity or activator composition, on the one hand, and the degree of differentiation of the tumors, the fraction of positive lymph nodes found, or the amount of estrogen-binding protein (or their molecular size) in the cytosol of the tumors, on the other. This apparent lack of correlation may reflect variations in local physiological conditions which necessitate the presence of widely differing amounts of activator in order to ensure a critical amount of the product of the reaction catalyzed by this enzyme, ie, plasmin. (Author abstract) (37 Refs)

14. CORRELATION OF CIRCULATING LEVELS OF A SERUM PROTEIN WITH TRIIODOTHYRONINE LEVELS AND HEPATOMA GROWTH.

Coetzee ML, Short J, Klein K, Ove P
(c/o Ove) Dept. Anatomy and Cell Biology, Univ. Pittsburgh, Sch. Medicine, Pittsburgh, PA, 15261
Cancer Res; 42(1):155-160 1982

In this communication, we provide evidence that proliferation of transplantable Morris hepatoma 7777 might to some extent be regulated by triiodothyronine and/or a specific serum protein, the levels of which are correlated with levels of triiodothyronine. The protein has an estimated mol wt of 80,000 and migrates as one band on polyacrylamide gel electrophoresis. In normal rats, this protein accounts for approx 1% of the total serum protein. Both the circulating levels of the serum protein and proliferation of transplanted hepatoma cells were decreased in thyroidectomized rats. Elevated levels of the serum protein and increased cell proliferation were observed when animals had 70% of their liver removed prior to transplant, were given injections of triiodothyronine, or had a 10-day-old first transplant surgically removed. Some evidence is also provided suggesting that the synthesis of the serum protein is stimulated by thyroid hormone. (Author abstract) (36 Refs)

15. FIBRONECTIN AND GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION IN NORMAL HUMAN BRAIN AND ANAPLASTIC HUMAN GLIOMAS.

Jones TR, Ruoslahti E, Schold SC, Bigner DD
(c/o Bigner) Dept. Pathology, Duke Univ. Medical Center, Durham, NC, 27710
Cancer Res; 42(1):168-177 1982

The expression of fibronectin (FN), an extracellular matrix glycoprotein with adhesive and opsonic properties, was examined in normal and neoplastic human CNS tissues. In both normal adult and fetal brain, FN expression, as detected by immunohistochemistry, was restricted to the vasculature. Neurons and glia did not stain. FN expression in 9 of 10 gliomas examined was qualitatively similar to that in normal fetal and adult brain in that it was confined to the blood vessels. Nevertheless, in one case, there was interstitial expression of FN. Four tumors exhibited varying degrees of fibroblastic overgrowth in which cells containing FN were detected. Frozen sections of eight athymic mouse-borne anaplastic human gliomas were stained simultaneously by a double-label fluorescein-rhodamine fluorescence technique for both FN and glial fibrillary acidic protein (GFAP), a glial and glioma marker. Seven of eight tumors expressed FN, seven of eight expressed GFAP, and six of eight expressed both. FN was detected as fine fibrils and specks between cells while GFAP presented as a homogeneous intracellular fluorescence. In many areas of the tumors, groups of cells expressed both FN and GFAP. Eleven human glioma-derived cell lines were examined for both FN and GFAP expression. In two cell lines, there were cells which contained both GFAP and FN simultaneously. Athymic mouse-borne tumors were developed by the injection of an FN-producing glioma-derived cell line into athymic mice. Human FN plasma levels were monitored, and some tumors larger than 1.0 g produced FN which was detectable in the plasma, thereby demonstrating that tumor-produced FN can be shed from human glioma-derived cells in sufficient amounts to be detectable in the bloodstream. These data show that FN expression in anaplastic human gliomas is seen with the greatest frequency in athymic mouse-borne tumors; GFAP-positive cultured cells are also capable of FN expression. The original tumors rarely express detectable amounts of FN. The biological basis for this heterogeneity of FN expression rests in the nature of the individual tissue studied, its cellular environment (ie, in vivo or in vitro), and its status with regard to neoplasia. (Author abstract) (47 Refs)

16. PARANUCLEAR MICROFILAMENTS IN MULTIPLE MYELOMA ASSOCIATED WITH THE PRESENCE OF F REE LIGHT CHAINS.

Arkin CF, Federman M, Gerson B
Lab. Pathology, New England Deaconess Hosp., Boston, MA, 02215
Ann Clin Lab Sci; 11(6):506-510 1981

The presence of microfilaments in fresh tissue (biopsy material, peripheral blood) from multiple myeloma patients (2 women; 75 and 78 yr old) was studied. In one patient, immunoelectrophoresis demonstrated free light chains in both serum and urine. The urine showed two distinct areas of restricted mobility, suggesting the presence of free lambda polymers. Electron microscopic studies demonstrated paranuclear microfilaments in both cases. The microfibrils may represent an overgrowth of normal cytoskeletal structures or of polymerized light chains; microfibrils are abnormal and may constitute a convenient marker in differentiating benign from malignant plasma cells. (21 Refs)

17. VASOACTIVE INTESTINAL POLYPEPTIDE MODULATION OF LYMPHOCYTE ADENYLATE CYCLASE.

O'Dorisio MS, Hermina NS, O'Dorisio TM, Balcerzak SP
Dept. Medicine, Ohio State Univ., 410 W. 10th Ave., Columbus, OH, 43210
J Immunol; 127(6):2551-2554 1981

Vasoactive intestinal polypeptide (VIP) was found to be a potent stimulator of lymphocyte adenylate cyclase activity. VIP-induced activation of adenylate cyclase was specific for lymphocytes among peripheral blood cells; ie, VIP did not stimulate the adenylate cyclase activity of neutrophils, monocytes, or platelets. The VIP-induced activation of lymphocyte adenylate cyclase was time, temperature, and concentration dependent. VIP and the GTP analog, Gpp(NH)p, acted synergistically to stimulate lymphocyte adenylate cyclase; stimulation by VIP and PGE1 was additive; and VIP activation was antagonized by somatostatin. VIP-mediated activation of lymphocyte adenylate cyclase was observed in normal human T cells, B cells obtained from a patient with chronic lymphocytic leukemia, and a human T cell culture line. The Raji human B cell culture line did possess adenylate cyclase activity, but this activity was not stimulated by VIP. These results suggest that lymphocytes possess functional receptors for VIP and that this peptide may play a role in modulation of lymphocyte function. (Author abstract) (41 Refs)

BINDING/ RECEPTOR PROTEINS

18. GLYCOLIPIDS: RECEPTORS FOR FIBRONECTIN?

Yamada KM, Kennedy DW, Grotendorst GR, Momoi T
Lab. Molecular Biology, NCI, Bethesda, MD, 20205
J Cell Physiol; 109(2):343-351 1981

Three different assays of fibronectin biological activity were examined for their sensitivity to inhibition by glycolipids. These assays measured cell-cell or cell-substrate adhesive interactions, and quantitate fibronectin's hemagglutinating activity, capacity to mediate cell spreading on plastic dishes, and restoration of normal alignment and morphology to transformed cells. Ganglioside s inhibited fibronectin-mediated hemagglutination, cell spreading, and restoration of a normal morphologic phenotype to transformed cells. Of the other lipids tested which were less effective inhibitors, the more negatively charged were the most effective. The most effective ganglioside inhibitors generally contained the most sialic acid residues. The oligosaccharide portions of gangliosides retained inhibitory activity; those with more sialic acid were more effective. These results are consistent with the notion that the 'receptor' for fibronectin on a variety of cell types contains a negatively charged lipid, eg, a ganglioside. Lipids other than gangliosides could conceivably serve as ligands for fibronectin interaction, depending on their concentration on the cell surface. The specificity for certain gangliosides is relative rather than absolute. There is no evidence that fibronectin needs a living cell for its ganglioside-inhibitable activity. Oligosaccharides on glycoproteins may not be fibronectin receptors, since purified glycopeptides did not inhibit hemagglutination. (32 Refs)

MEMBRANE/ SURFACE PROTEINS

19. NEGATIVE SURFACE CHARGE OF ABNORMAL HUMAN TROPHOBLAST.

Kawagoe K, Kawana T, Sakamoto S
Dept. Obstetrics Gynecology, Faculty Medicine, Univ. Tokyo, Tokyo 113, Japan
Acta Histochem Cytochem; 14(5):554-560 1981

The negative surface charge of the abnormal human trophoblast was characterized using cationized ferritin as an ultrastructural marker. In addition, the relationship between negative surface charge and surface glycoproteins was examined by ruthenium red staining. In human placental villi from cases of spontaneous abortion and cases of partial mole, the number of cationized ferritin particles on the trophoblastic surface generally decreased. In villi of complete hydatidiform moles, the number of ferritin particles on the trophoblastic surface was slightly increased, and the distribution pattern of the negative surface charge was similar to that of normal villi. (22 Refs)

20. MOLECULAR COMPLEXITY OF LEUKOCYTE SURFACE GLYCOPROTEINS RELATED TO THE MACROPHAGE DIFFERENTIATION ANTIGEN MAC-1.

Trowbridge IS, Omary MB
Dept. Cancer Biology, Salk Inst. for Biological Studies, San Diego, CA, 92138
J Exp Med; 154(5):1517-1524 1981

A differentiation antigen designated Mac-1 was identified on murine macrophages using the monoclonal antibody M1/70. Mac-1 belongs to a family of structurally and antigenically related glycoproteins found on a variety of hemopoietic cells including phagocytes and other cell types. The functional significance of these glycoproteins is discussed. (24 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

21. COLLAGEN SYNTHESIS IN CELLS CULTURED FROM V. RECKLINGHAUSEN'S NEUROFIBROMATOSIS.

Peltonen J, Marttala T, Vihersaari T, Renvall S, Penttinen R
(c/o Penttinen) Dept. Medical Chemistry, Univ. Turku, Kiinamyllynkatu 10, SF-20520 Turku 52, Finland
Acta Neuropathol (Berl); 55(3):183-187 1981

Cultured neurofibromatosis cells (NC) from tumors in two patients with von Recklinghausen's disease synthesized relatively much more collagen than cultures of skin fibroblasts of the same patients or of healthy controls. Further biochemical studies in cultures of one patient indicated that most of the collagenous proteins resembled Type I collagen. The increased growth potential observed and high relative collagen synthesis in NC may explain the appearance of tumors. (26 Refs)

22. IN VITRO TRANSLATION OF HYPOXANTHINE/ GUANINE PHOSPHORIBOSYLTRANSFERASE MRNA: CHARACTERIZATION OF A MOUSE NEUROBLASTOMA CELL LINE THAT HAS ELEVATED LEVELS OF HYPOXANTHINE/GUANINE PHOSPHORIBOSYLTRANSFERASE PROTEIN.

Melton DW, Konecki DS, Ledbetter DH, Hejtmancik JF, Caskey CT
Howard Hughes Medical Inst. Lab., Dept. Medicine, Baylor Coll. Medicine, Houston, TX, 77030
Proc Natl Acad Sci USA; 78(11,part2):6977-6980 1981

Antibody specific for the native form of Chinese hamster hypoxanthine/guanine phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) was used to detect the synthesis of HPRT protein in a rabbit reticulocyte lysate translation system primed with mRNA from Chinese hamster tissues and cultured cells. Electrophoretic analysis of the immunopurified products from the translation of mRNA from wild-type and a series of mutant Chinese hamster cells indicated that HPRT synthesis in vitro qualitatively and quantitatively corresponded to synthesis in vivo. The translation system was used to identify two mRNA sources producing high levels of HPRT protein: Chinese hamster brain and a mouse neuroblastoma HPRT revertant cell line, NBR4. Translation of NBR4 mRNA generated 25-50x more HPRT protein than mRNA from wild-type cells. The basis for HRPT overproduction is considered in view of an X chromosome alteration found in NBR4 cells. (Author abstract) (20 Refs)

23. LIPOPROTEIN APOLIPOPROTEIN SYNTHESIS BY HUMAN HEPATOMA CELLS IN CULTURE.

Rash JM, Rothblat GH, Sparks CE
(c/o Rothblat) Dept. Physiology and Biochemistry, Medical Coll. Pennsylvania, Philadelphia, PA, 19129
Biochim Biophys Acta; 666(2):294-298 1981

Lipoprotein synthesis was demonstrated by double diffusion with low density lipoprotein antibody, and by 3H-labeled amino acid incorporation into proteins of the d less than 1.063 g/ml centrifugally isolated lipoprotein fraction. Radioactive label was incorporated predominantly into apolipoprotein B (60%), apolipoprotein A-I (20%) and apolipoprotein C (12%), as determined by Sepharose column chromatography and polyacrylamide gel electrophoresis. Incorporation of radioactive label into apol ipoprotein B was inhibited by the presence of albumin in the medium, and was restored to control levels with the addition of 1 mM oleic acid, indicating that cell synthesis of apolipoproteins could be modified by culture conditions. The human hepatoma cell line, Hep G2, provides a potential in vitro model for the study of regulation of human hepatic lipoprotein and apolipoprotein synthesis. (Author abstract) (16 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

24. INHIBITORY EFFECTS OF FOUR ISOABRINS ON THE GROWTH OF SARCOMA 180 CELLS.

Lin JY, Lee TC, Tung TC
Dept. Biochemistry, Coll. Medicine, Natl. Taiwan Univ., Taiwan, Republic of China
Cancer Res; 42(1):276-279 1982

The four isoabrins were shown to be capable of inhibiting the growth of tumor cells in vivo when one-fifth of their median lethal dose was used. From the in vitro experiments, the doses required for 50% inhibition of protein biosynthesis are 3.2 pg, 45 ng, 32 ng, and 10 ng/ml for abrin-a, -b, -c, and -d, respectively. Except for abrin-b, a good correlation between the inhibitory effects of abrins on the tumor growth and protein biosynthesis was observed. These isoabrins show a moderate inhibitory effect of DNA biosynthesis. (Author abstract) (19 Refs)

25. PHORBOL ESTER-INDUCED ANCHORAGE INDEPENDENCE AND ITS ANTAGONISM BY RETINOIC ACID CORRELATES WITH ALTERED EXPRESSION OF SPECIFIC GLYCOPROTEINS.

Dion LD, De Luca LM, Colburn NH
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Carcinogenesis; 2(10):951-958 1981

Biochemical studies in JB-6 mouse epidermal cells were carried out to determine whether specific changes in glycoprotein synthesis might be targets for tumor-promoting action of phorbol esters and for anti-promoting action of retinoids. Sodium dodecylsulfate-polyacrylamide gel electrophoresis profiles of cells treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) at concentrations inducing anchorage independence in JB-6 cells (1-100 nanog/ml) showed substantial decreases in mannose, fucose, glucosamine, and galactose incorporation into glycoconjugates of approx 180,000 (gp 180), 150,000 (gp 150), and 220,000 (gp 220) daltons mol wt. Glycoprotein labeling was unaffected by TPA at 0.1 nanog/ml, a concentration which does not induce anchorage independence. Two TPA analogues, phorbol didecanoate and phorbol dibenzoate, reduced labeled mannose incorporation into the three glycoproteins, whereas phorbol and phorbol diacetate, which do not promote anchorage independence, had no effect. Retinoic acid (10(-6) M), a potent inhibitor of TPA-induced anchorage independence in JB-6 cells, enhanced mannose and leucine labeling of gp180 and gp150 and antagonized the TPA effect, suggesting that decreased gp180 and gp150 synthesis could be required in late-stage promotion. (22 Refs)

26. TUMOR PROMOTERS INDUCE THE SYNTHESIS OF A SECRETED GLYCOPROTEIN IN MOUSE SKIN AND CULTURED PRIMARY MOUSE EPIDERMAL CELLS.

Gottesman MM, Yuspa SH
Lab. Molecular Biology, NCI, NIH, Bethesda, MD, 20205
Carcinogenesis; 2(10):971-976 1981

Effects of tumor promoters and anti-promoters on synthesis and secretion of a 35,000 dalton transformation-dependent secreted glycoprotein (major excreted protein, MEP) by primary mouse epidermal cells were investigated. 12-O-Tetradecanoylphorbol-13-acetate (TPA) increased synthesis and secretion of MEP, an effect that was maximal at 6 hr. This time course is consistent with an effect of TPA on transcription of MEP messenger RNA. The induction of MEP synthesis was very sensitive to TPA, with half-maximal stimulation (at 3 nanog/ml or less). TPA also induced MEP synthesis in mouse skin in situ. The anti-promoters lidocaine and retinoic acid had no effect on basal MEP synthesis or secretion, or on TPA stimulation. Fluocinolone acetonide decreased basal levels of MEP synthesis without blocking TPA-induced MEP synthesis. The data suggest that the effects of the three anti-promoters are not mediated via an alteration in MEP synthesis or secretion and that an increase in MEP synthesis per se is not sufficient for promotion. (33 Refs)

DIETARY AND NUTRITIONAL EFFECTS

27. DISORDERS OF MEMBRANE DIGESTION OF PROTEINS IN PATIENTS WITH OBSTRUCTIVE JAUNDICE OF TUMORAL ETIOLOGY.

Gorbunov OM
Dept. Hospital Surgery, D. I. Ul'ianov Medical Inst., Kuibyshev, USSR
Klin Khir; (9):56-57 1981

Activities of small intestine dipeptidases were studied in 7 patients with cancer of the papilla of Vater, in 42 with pancreatic cancer, and in 8 controls. Analyses of surgical specimens of jejunum showed decreases in glycylleucine dipeptidase activity in specimens from all patients that had obstructive jaundice of tumoral etiology. Radical surgery brought about some normalization of membrane digestion. (no Refs) (In RUS)

28. PROTEIN NUTRITION IN CLINICAL PRACTICE.

Jeejeebhoy KN
Dept. Medicine, Univ. Toronto, Toronto, Canada, Canada
Br Med Bull; 37(1):11-17 1981

Protein metabolism may be altered due to insufficient intake, altered metabolism (including neoplastic disorders), specific plasma protein deficiency, or reduced protein synthesis. Causes, assessment, and treatment of protein disorders are discussed. Protein and amino acid metabolism are described. (74 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

29. THE RELATIONSHIP BETWEEN POLYAMINE VALUE AND STAGE CLASSIFICATION IN PATIENTS WITH CANCER OF THE LARGE INTESTINE (MEETING ABSTRACT).

Miyata J, Takami H, Yamamoto O, Nakata M, Oqihara H, Teramoto T, Kodaira S, Abe O
Dept. Surgery, Sch. Medicine, Keio Univ., Shinjuku-ku, Tokyo 160, Japan
Geka To Taisha Eiyo; 15(Special):62 1981

The preoperative concentration of polyamines in the serum and RBC of 28 patients with cancer of the large intestine (1 cecum, 1 ascending colon, 3 transverse colon, 3 descending colon, 10 sigmoid colon, 9 rectum, 1 proctos) was measured. The blood was collected early in the morning before breakfast, centrifuged, and separated into serum and RBC. After its protein was removed by using trichloroacetic acid (TCA), it was hydrolyzed with 6 N HCl. The TCA was removed with ether, and the polyamines were measured by high performance liquid chromatography. The concentration of putrescine (PUT) in the serum and RBC tended to decrease but that of spermidine (SPD) and spermine (SPM) tended to increase with the advance in the stage according to the classification by Duke, by Aster-Coller and in the General Rules for the Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus alike. The SPD/SPM ratio in the RBC of patients with Duke's classification A and D was 2.207 and 1.117, respectively (p less than 0.001), there being a clear decrease with the advance in the stage. The concentration of the polyamines in the serum showed a similar tendency. Of the various factors showing the pathohistological degree of advance, that is hepatic metastasis (H), serosal invasion (S), peritoneal metastasis (P), number of nodes invaded (N), lymphatic invasion (ly) and vascular invasion (v), H and S greatly affected the concentration of the polyamines but v showed no relation to its concentration. The SPD/SPM ratio for well-differentiated and moderately differentiated adenocarcinoma according to Duke's classification was 2.414 and 1.653, respectively, there being a significant difference (p less than 0.05) between the two groups. Therefore, it was considered meaningful to add the concentration of the polyamines (PUT, SPD, SPM) and SPD/SPM ratio to the pathohistological classification of the advance of the cancer. (Author abstract) (no Refs) (In JPN)

30. LEVELS OF POLYAMINES AND NUCLEIC ACIDS IN HUMAN BREAST CARCINOMA.

Romano M, Cecco L, Cerra M
Istituto Tumori, Via Mariano Semmola, 80131 Napoli, Italia
Tumori; 67(5):431-435 1981

Polyamine and nucleic acid levels were determined in normal human breasts, in primary infiltrating ductal carcinoma breasts, in the uninvolved tissues of the same carcinoma-bearing breasts and in the urine of the same patients. The results showed that the production of polyamines in the carcinoma tissue is significantly higher than in normal tissue; the presence of a carcinoma in the breast does not modify the levels of polyamines and nucleic acids in the surrounding uninvolved tissue; spermidine and spermine are correlated both with DNA and RNA in the carcinoma; the urinary polyamine levels in the carcinoma-bearing patients were not significantly different from that of controls. There appeared to be a close relationship between polyamine biosynthesis and tumoral mammary cell development and a very fine control between the biosynthesis of both spermidine and spermine and nucleic acids. In addition, the localized solid breast carcinoma, with non-proven metastases, did not provoke an increase in polyamine excretion. (Author abstract) (25 Refs)

OTHER RELATED STUDIES

31. RELATION OF ACOUSTIC CHARACTERISTICS OF HUMAN GASTRIC JUICE TO ITS COMPOSITION FOR SOME STOMACH DISEASES.

Klemin VA, Karev ID, Sarvazyan AP, Timokhina LM, Ruchkin VV, Mayorov EA
(c/o Sarvazyan) Inst. Biological Physics, Acad. Sciences USSR, 292 Puscino, Moscow Region 142, USSR
Stud Biophys; 84(2):139-144 1981

The acoustic characteristics of gastric juice from patients with gastritis (48), gastric ulcers (84), and gastric cancer (68) were studied; the relationship to changes in K+, Ca++, Na+, protein, and polypeptide levels was examined. Absorption of ultrasound was shown to be determined by protein concentration. Ultrasound velocity was determined by K+, Ca++, Na+, polypeptide, and protein levels and was dependent on pathology type. (7 Refs)

32. SENSITIVITY IN DETECTION OF MONOCLONAL IMMUNOGLOBULINS BY THIN-LAYER ISOELECTRIC FOCUSING AND CERTAIN ELECTROPHORESES IN LABORATORY USE.

Kamiya-Harada Y, Sato Y, Otani H, Kawakami M
Molecular Biology Lab., Sch. Medicine, Kitasato Univ., Kitasato, Sagamihara, Kanagawa 228, Japan
Microbiol Immunol; 25(10):1067-1076 1981

The best hydrogen ion concentration range for detection of the band group specific for monoclonal immunoglobulin (MCIG) by thin-layer isoelectric focusing (IEF) was pH 5 to 8. Concentration of MCIG was estimated from the intensity of band staining. IEF was performed with patient sera containing myeloma proteins of various classes using pH gradient gels of different pH ranges. Lowest concentration of MCIG detected was approx 0.1 mg/ml, which was 8-32 times lower than the concentration detectable by cellulose acetate membrane electrophoresis (CAE) or immunoelectrophoresis (IE) in routine laboratory use. Intensities of the band stain in IEF showed a linear relationship with concentrations from 0.1-50.0 mg/ml. Blind tests on sera resulted in some disagreement between the concentration of MCIG from results with CAE and IE and those determined by IEF. Results suggested that IEF will be a useful method to detect early malignant monoclonal immunoglobulinemia after the technique and equipment are improved for laboratory tests. (17 Refs)

33. URINARY EXCRETION OF GLYCOSAMINOGLYCANS AND HYDROXYPROLINE IN PAGET'S DISEASE OF BONE, COMPARED WITH NEOPLASTIC INVASION OF BONE.

Bower L, Manley G
Dept. Chemical Pathology, Torbay Hosp., Torquay, Devon, England
J Clin Pathol; 34(10):1097-1101 1981

Urinary glycosaminoglycan and hydroxyproline excretion was studied in 11 patients with clear evidence of Paget's disease of bone. Urinary hydroxyproline, cetyl pyridinium chloride (CPC)-precipitable uronic acid and CPC-precipitable hexosamine were expressed as ratios to urinary creatinine. Urine samples were concentrated 1,000x by vacuum dialysis and the glycosaminoglycans examined by electrophoresis on cellulose acetate followed by staining with alcian blue. All the cases studied showed markedly raised hydroxyproline excretion, whereas the uronic acid excretion was normal or only slightly raised in 10 of the 11 cases studied. One patient who had a raised uronic acid and raised hydroxyproline concentration was shown to have osteosarcoma as a complication of Paget's disease. The very high hydroxyproline:creatinine ratio in all cases of Paget's disease (mean 241.8 mmol hydroxyproline/mol creatinine) contrasted sharply with the cases of disseminated neoplasm, where the ratio was either normal or slightly raised (mean 29.3 mmol hydroxyproline/mol creatinine). The ratio of hydroxyproline to CPC-precipitable uronic acid was also markedly raised in cases of Paget's disease (mean 77.3 mmol hydroxyproline/mmol uronic acid) and was lower in the neoplastic group (mean 14.1 mmol hydroxyproline/mmol uronic acid) but showed no advantage over the hydroxyproline:creatinine ratio in differentiating the two groups. The urinary hydroxyproline:creatinine ratio promises to be of value in differentiating between Paget's disease of bone and neoplastic invasion of bone. A marked rise in CPC-precipitable uronic acid excretion alone is more suggestive of neoplastic invasion of bone, and if associated with a marked increase in hydroxyproline excretion, it raises the possibility of neoplastic change in Paget's disease of bone. The results of this study also suggest that bone collagen, rather than bone tissue in general, is primarily affected in Paget's disease. (Author abstract) (17 Refs)

34. REQUIREMENT OF GSH FOR L-METHIONINE TRANSPORT, AND GAMMA-GLUTAMYLTRANSFERASE ACTIVITY OF EHRLICH ASCITES TUMOR CELLS.

Shiozaki H, Hirasawa M, Oribe N, Ohno T, Furukawa M, Higashi R, Higashi T, Sakamoto Y

(c/o T. Higashi) Dept. Biochemistry, Inst. for Cancer Res., Osaka Univ. Medical Sch., 1-1-50 Fukushima, Fukushimna-ku, Osaka 553, Japan

Med J Osaka Univ; 31(3/4):67-73 1981

The possible association of gamma-glutamyltransferase (g-GT) with L-methionine transport in Ehrlich ascites tumor was studied in relation to the role of glutathione (GSH). Methionine uptake was suppressed by GSH oxidizing agents (diamide and azoester), by a gamma-glutamylcysteine synthetase inhibitor (methionine sulfoximine), and by g-GT inhibitors (6-diazo-5-oxo-L-norleucine and maleate). These findings showed that GSH was required for methionine uptake and suggested that its action was mediated by g-GT. However, further studies indicated that the enzyme does not play a major role in methionine uptake by Ehrlich ascites cells. Cellular methionine uptake was 1.98 nanomoles (nmol)/min/mg/protein, but g-GT activity was a low as 0.22 nmol of p-nitroaniline formed/min/mg/protein in the presence of glycine. In addition, another strain of Ehrlich tumor cells with higher enzyme activity showed slower methionine uptake. (36 Refs)

35. EFFECTS OF LIPOSOME STRUCTURE AND LIPID COMPOSITION ON THE ACTIVATION OF THE TUMORICIDAL PROPERTIES OF MACROPHAGES BY LIPOSOMES CONTAINING MURAMYL DIPEPTIDE.

Schroit AJ, Fidler IJ

Cancer Metastasis and Treament Lab., Natl. Cancer Inst. Frederick Res. Center, Frederick, MD, 21701

Cancer Res; 42(1):161-167 1982

Various vesicle structures and lipid compositions have been studied to identify the optimal type of liposome for delivery of the macrophage-activating agent muramyl dipeptide (MDP) to macrophages. Evaluation of the ability of liposomes to be phagocytosed by macrophages established that optimal initial rates of engulfment were obtained when multilamellar vesicles (MCV) composed of distearoylphosphatidylcholine (18:0 PC): phosphatidylserine (PS) (7:3 mol ratio) were used. MLV composed exclusively of 18:0 PC were phagocytosed at rates greater than or equal to those of MLV composed of egg phosphatidylcholine (PC):PS, whereas MLV composed only of egg PC were very poorly phagocytosed. Although phagocytosis was enhanced by incorporation of PS into MLV, the inclusion of PS brought about significant enhancement in liposome permeability in the presence of serum. The inclusion of PS, however, was a requirement for the delivery of MLV to the lungs following iv injection into mice whether used in conjunction with 18:0 PC or egg PC. Activation of macrophages to become tumoricidal against syngeneic tumor cells with liposome-encapsulated MDP was superior in both degree and duration when MLV composed of 18:0 PC:PS (7.3 mol ratio) were used. MLV were found to be superior to large unilamellar vesicles containing equal amounts of lipid and entrapped MDP. On the other hand, higher levels of macrophage activation were obtained when an equivalent amount of a lipophilic MDP derivative, muramyl tripeptide:phosphatidylethanolamine, was incorporated into the liposome bilayer irrespective of whether the adjuvant was incorporated in liposomes composed of 18:0 PC:PS or egg PC:PS. (Author abstract) (42 Refs)

36. INFLUENCE OF SERUM AND AMINO ACIDS ON THE ACCUMULATION OF AMINOISOBUTYRATE BY RAT HEPATOMA CELLS. A DEDIFFERENTIATION OF TRANSPORT ROUTES?

Gulumian M, Manchester KL

Natl. Centre Occupational Health, Johannesburg 2001, South Africa

Biochim Biophys Acta; 649(1):24-30 1981

Rat hepatoma cells accumulate considerably less 2-aminoisobutyrate after cultivating in the absence of serum - the change in rate of aminoisobutyrate uptake takes place within 1 hr of serum starvation. Starvation of amino acids by contrast raises aminoisobutyrate uptake in the presence or absence of serum, but the cells are much less responsive to amino acid supply than to availability of serum. Phosphate (10 mM) reduced aminoisobutyrate uptake by cells grown in serum to that exhibited by serum-starved cells. Aminoisobutyrate uptake by cells grown in serum was reduced by glycine, proline, alanine, serine, glutamine, metyhylaminoisobutyrate and 2-aminonorbornane-2-carboxylate, the effects of methylaminoisobutyrate and 2-aminonorbornane-2-carboxylate being additive. However, similar inhibition phenomena were not seen for cells deprived of serum where aminoisobutyrate uptake tended to a relatively constant level insensitive to inhibitory influences, yet substantially greater than that arising by simple diffusion. The comparative insensitivity of our hepatoma line when starved of serum to competition and repression phenomena is in contrast

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to findings of others. Our results also suggest a lack of clear delineation of specificities for the A and L transport systems as usually defined. (Author abstract) (17 Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Abe O 29
Anderson KM 5
Arkin CF 16
Balcerzak SP 17
Banks MA 11
Baranowski J 5
Bayreuther K 4
Berlov GA 3
Bignami A 4
Bigner DD 15
Bloom SR 8
Bower L 33
Burnett D 7
Bykowska K 10
Camiolo SM 13
Caskey CT 22
Cecco L 30
Cerra M 30
Chandy KG 7
Coetzee ML 14
Colburn NH 25
Collen D 10
Crockson RA 7
Dad'kov IG 3
Dahl D 4
De Luca LM 25
Dion LD 25
Economou SG 5
Eppenberger U 2
Ershova-Pavlova 3
Evers JL 13
Fabbro D 2
Federman M 16
Fidler IJ 35
French FS 6
Furukawa M 34
Gerson B 16
Ghatei MA 8
Gorbunov OM 27
Gottesman MM 26
Greenwood MA 11
Grotendorst GR 18
Gulumian M 36
Hejtmancik JF 22
Hermina NS 17
Higashi R 34
Higashi T 34
Hirasawa M 34
Hobika GH 13
Jeejeebhoy KN 28
Jones TR 15
Jungmann RA 2
Kamiya-Harada Y 32
Karev ID 31
Kawagoe K 19
Kawakami M 32
Kawana T 19
Kennedy DW 18
Klein K 14
Klemin VA 31
Kodaira S 29
Konecki DS 22
Kornblith PL 11
Krivchik AA 3
Laverson S 11
Ledbetter DH 22
Lee TC 24
Lee YC 8
Leonard RC 7
Lin JY 24
Ludlum DB 1
Ludwig-Festl M 4
Maclennan IC 7
Madeja JM 13
Manchester KL 36
Manley G 33
Markus G 13
Marttala T 21
Mayorov EA 31
McKeever PE 11
Mehta JR 1
Melton DW 22
Metcalf D 9
Miyata J 29
Momoi T 18
Moolten FL 12
Nakata M 29
Nicola NA 9
O'Dorisio MS 17
O'Dorisio TM 17
O'Shaugnessy D 8
Oh SK 12
Ohno T 34
Omary MB 20
Oqihara H 29
Oribe N 34
Osborn M 4
Otani H 32
Ove P 14
Patel J 13
Peltonen J 21
Penttinen R 21
Petrusz P 6
Quindlen E 11
Rash JM 23
Renard A 1
Renvall S 21
Rijken DC 10
Romano M 30
Rothblat GH 23
Ruchkin VV 31
Ruoslahti E 15
Sakamoto S 19
Sakamoto Y 34
Sarvazyan AP 31
Sato Y 32
Schneider SL 13
Schold SC 15
Schroit AJ 35
Shiozaki H 34
Short J 14
Smith B 11
Sparks CE 23
Stockley RA 7
Takami H 29
Teramoto T 29
Timokhina LM 31
Trowbridge IS 20
Tung TC 24
Verly WG 1
Vihersaari T 21
Weber K 4
Williams U 11
Wilson EM 6
Wood JR 8
Wood SM 8
Yamada KM 18
Yamamoto O 29
Yuspa SH 26

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In this issue:

- **NUCLEOPROTEINS** 1-4
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** 5-16
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** 17-38
- **BINDING/ RECEPTOR PROTEINS** 39
- **MEMBRANE/ SURFACE PROTEINS** 40-44
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** 45-50
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** 51-56
- **DIETARY AND NUTRITIONAL EFFECTS** 57-60
- **POLYAMINES - SYNTHESIS AND METABOLISM** 61-63
- **OTHER RELATED STUDIES** 64-71

NUCLEOPROTEINS

1. SEPARATION OF BASAL HISTONE SYNTHESIS FROM S-PHASE HISTONE SYNTHESIS IN DIVIDING CELLS.

Wu RS, Bonner WM
Lab. Molecular Pharmacology, Developmental Therapeutics Program, NCI, Bethesda, MD, 20205
Cell; 27(2):321-330 1981

Histone synthesis in cycling tissue-culture cells can be separated into basal synthesis and S-phase synthesis. S-phase histone synthesis comprises about 90% of total histone synthesis, and in this regard our results support the generally held model

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that most histone synthesis is tightly linked to DNA synthesis. Basal histone synthesis is less than 10% of the S-phase synthesis but persists throughout the S, G2 and G1 phases of the cell cycle, while S-phase synthesis rises in parallel with the increase in DNA synthesis. Chinese hamster ovary cells, the H2A variants Z and X, and the H3 variant 3, participate in basal histone synthesis while the H2A variants 1 and 2, and the H3 variant 2, participate in S-phase histone synthesis. H2B and H4 participate in both kinds of synthesis. Because specific H2A and H3 variants are involved, basal histone synthesis cannot be attributed to contamination by cells in S-phase. Whole cells and isolated nuclei yield the same results. Basal histone synthesis can be demonstrated in cells synchronized by mitotic shake-off either alone or in combination with isoleucine starvation and colcemid blockage. It is also differentiated from S-phase histone synthesis by its relative insensitivity to hydroxyurea, indicating that basal histone synthesis is not linked or is at least less tightly linked to DNA synthesis. The basal H2A variants X and Z of five other cell lines all show similar relative insensitivities to hydroxyurea treatment. These lines include attached cultures, suspension cultures, a normal line and transformed lines from human, mouse, rat and Chinese hamster, indicating that basal histone synthesis may be a property of all cycling cells. The integration of basal histones into chromatin seems to follow the same pathway as S-phase histones, but the kinetics are somewhat slower. (Author abstract) (32 Refs)

2. POLY ADP-RIBOSYLATION AS A PROSPECTIVE MODULATOR OF CHROMATIN STRUCTURE IN THE HELA CELL NUCLEUS.

Jump DB
Georgetown University Medical Center, Washington, DC
Diss Abstr Int [B]; 42(5):1858-B 1981

Several critical issues dealing with understanding the function of poly (ADP-Rib) polymerase in the HeLa nucleus have been addressed: (a) purification and characterization of poly (ADP-Rib) polymerase from HeLa nuclei; (b) determining that histone modification is most prominent during periods of DNA synthesis; (c) distribution of the enzyme and its acceptor proteins in chromatin with particular emphasis on the elucidation of the association of these proteins with extended regions of chromatin undergoing DNA synthesis; and (d) preliminary evidence indicating that poly ADP-ribosylation may function to promote condensation of modified domains resulting in decrease accessibility of chromatin DNA to nucleases. (no Refs)

3. TUMOR-ASSOCIATED CHROMATIN ANTIGENS OF HUMAN COLON ADENOCARCINOMA CELL LINES HT-29 AND LOVO.

Duhl DM, Banjar Z, Briggs RC, Page DL, Hnilica LS
Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
Cancer Res; 42(2):594-600 1982

Antiserum to dehistonized chromatin from human colon adenocarcinoma cell line HT -29 was raised in rabbits and used for the immunodetection of colon tumor-associated nuclear antigens. Quantitative microcomplement-fixation studies indicated that the antiserum had reactivity similar to those of chromatins of two human colon adenocarcinoma cell lines (HT-29 and LoVo) and with nonneoplastic colon. Immunocytochemical localization showed the chromatin antigens to be evenly dispersed throughout the nucleus. Immunochemical staining of electrophoretically separated chromosomal proteins transferred to nitrocellulose sheets revealed several intensely staining antigens predominantly in the mol wt range of 62,000 to 75,000. When the antiserum was immunoabsorbed with nonneoplastic human colon chromatin, two antigens with mol wt of 67,000 and 92,000 were identified only with the tumor chromatins. Antiserum immunoabsorbed with either LoVo or HT-29 chromatin failed to show any immunoreactivity, suggesting that these tumor-associated proteins, or antigenic sites residing on these proteins, are identical in both colon tumor cell lines. (Author abstract) (43 Refs)

4. A NUCLEAR ANTIGEN ASSOCIATED WITH CELL PROLIFERATION AND BLAST TRANSFORMATION. ITS DISTRIBUTION IN SYNCHRONIZED CELLS.

Takasaki Y, Deng JS, Tan EM

Div. Rheumatic Diseases, Dept. Medicine, Univ. Colorado Sch. Medicine, Denver, CO, 80262

J Exp Med; 154(6):1899-1909 1981

The time relationship of nuclear antigen present in proliferating cells (PCNA) to synthesis of DNA, RNA, and protein during the cell cycle was examined. Two sera (EB and MN) from patients with systemic lupus erythematosus contained precipitating anti-PCNA antibody along with antibodies to other nuclear antigens. Insolubilized rabbit kidney extract was used as an immunoadsorbant; precipitating antibodies to other nuclear antigens were removed with the exception of anti-PCNA. WiL-2 diploid human B lymphocytes were examined with EB-absorbed serum at different phases of synchronized growth. At G0, immediately after release from density-dependent arrest, PCNA were present as speckled nucleoplasmic staining. At 12 hr, nucleolar staining and speckled nucleoplasmic staining were evident. At 20 hr, nucleolar staining had disappeared from most cells, but speckled nucleoplasmic staining was strong. In mitosis, staining was more intense in areas peripheral to chromosome condensation, but radiating strands of staining could be seen within the region of chromosome condensation. In WiL-2 cells, nucleolar staining predominated in a period which overlapped late G1 and early S, while in phytohemagglutinin-stimulated lymphocytes, nucleolar staining was the dominant pattern well before DNA synthesis occurred. Results demonstrate that the relationship of PCNA to proliferation and blast transformation may be associated with events related to DNA synthesis in the cells. (14 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

5. ONE BETA-TUBULIN SUBUNIT ACCUMULATES DURING NEURITE OUTGROWTH IN MOUSE NEUROBLASTOMA CELLS.

Edde B, Jeantet C, Gros F

Laboratoire de Biochimie Cellulaire, College de France, 75231 Paris Cedex 05, France

Biochem Biophys Res Commun; 103(3):1035-1043 1981

Tubulin heterogeneity was analyzed during morphological differentiation of the mouse neuroblastoma clone C 1300. Beta2-tubulin was found to be specific for the differentiated cells. Synthesis of this isoform is strictly related to the neurite outgrowth process and has been shown to be regulated at the post-translational level. (22 Refs)

6. PHOSPHORYLATION OF RIBOSOMAL PROTEIN S6 IN THE EHRLICH ASCITES TUMOR CELL: LACK OF EFFECT OF PHOSPHORYLATION UPON RIBOSOMAL FUNCTION IN VITRO.

Mastropaolo W, Henshaw EC
(c/o Henshaw), Univ. Rochester Cancer Center, Rochester, NY, 14642
Biochim Biophys Acta; 656(2):246-255 1981

The incorporation of 32Pi into the ribosomal protein S6, which is a component of the 40 S ribosomal subunit, was measured in Ehrlich ascites tumor cells growing in suspension culture. During a 2-hr incubation period, incorporation was 10-fold greater into S6 of cells growing exponentially in complete medium than in cells in which protein synthesis and growth were inhibited by omission of glutamine from the medium. Since the labeling of other phosphoproteins was not similarly depressed in glutamine-deprived cells, the decreased labeling most likely reflects a specific decrease in phosphate incorporation into S6 rather than a lower phosphate pool specific activity. The cycling of ribosomes is inhibited in glutamine-deprived cells, with an accumulation of monomeric ribosomes indicating an inhibition of protein synthesis initiation. To assess whether the decreased phosphorylation of S6 in the ribosomes of glutamine-deprived cells was responsible for their decreased ability to initiate protein synthesis and enter polyribosomes, a mixture of 14C-labeled ribosomes from rapidly growing cells and 3H-labeled ribosomes from glutamine-deprived cells was added to a rabbit reticulocyte lysate cell-free protein synthesizing system in which ribosomes cycle actively. The 14C/3H ratio was determined in polyribosomes as a measure of the relative ability of the two kinds of ribosomes to initiate protein synthesis. The 14C/3H ratio in polyribosomes was found to be identical to the input of labeled ribosomes, indicating an equal ability of ribosomes from growing cells and glutamine-deprived cells to function in initiation. Control studies indicated lack of significant dephosphorylation of S6 during purification of ribosomes and during incubation in the reticulocyte lysate. Thus, a functional difference between more and less phosphorylated ribosomes could not be demonstrated in this system. (Author abstract) (34 Refs)

7. ACYLTRANSFERASE ACTIVITY AND CYTOCHEMICAL LOCALIZATION IN DIFFERENTIATING NEUROBLASTOMA.

Williams LR, Sandra A, Sandquist D, Williams TH
Neuroscience Lab. Building, Univ. Michigan, 1103 E. Huron, Ann Arbor, MI, 48109
Cell Differ; 10(5):289-298 1981

Acyltransferase (AT) activity was assayed biochemically and localized cytochemically in neuroblastoma monolayers. AT activity increased linearly with increasing amounts of protein and was a hyperbolic function of palmitoyl-coenzyme A concentration. Concentrations of 125 uM palmitoyl-coenzyme A were used routinely for correlation with cytochemical results. Neurite growth, but not AT activity, was induced by 24 hr exposure to serum-free medium or to 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724). Neurite growth was induced and AT activity increased two-fold after 4-day exposure to Ro20-1724 or dexamethasone (Dex). Dex treatment caused a small decrease in glycerol-3-phosphate incorporation into lysophosphatidic and phosphatidic acid and a corresponding increase in incorporation into neutral lipids. Twice as much triacylglycerol was synthesized as in an alcohol control. Two additional unidentified polar glycerides appeared in trace amounts after Dex treatment. AT was localized in mitochondria. Although increased AT activity correlates with Dex-induced morphological changes in neuroblastoma cells, AT activity and neurite extension may not be causally related. (52 Refs)

8. ISOLATION AND CHARACTERIZATION OF AN ESTER HYDROLASE ACTIVE ON PHORBOL DIESTERS FROM MURINE LIVER.

Shoyab M, Warren TC, Todaro GJ
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
J Biol Chem; 256(23):12529-12534 1981

An esterase which cleaves the 12-ester group of phorbol-12,13-diesters has been purified to electrophoretic homogeneity from murine liver cytosol using ammonium sulfate fractionation, Sephadex G-200 gel filtration, Con A-Sepharose chromatography, and phenyl-Sepharose chromatography. the enzyme is a single chain, hydrophobic glycoprotein with a mol wt of 60,000 and exhibits optimum activity at pH 7.5 to 8.5. The hydrolase has an isoelectric point (pI) of 5. The enzyme is heat- and acid-labile. Zinc, cobalt, and fluoride ions inhibit its activity. Phenylmethylsulfonyl fluoride is a potent inhibitor of the hydrolase. Sarkosyl also inhibits the enzyme at millimolar concentrations. The enzyme inactivates biologically active phorbol-12,13-diesters in a dose-, time-, and temperature-dependent manner. The enzyme inhibits phorbol-12,13-dibutyrate binding to its receptor in a noncompetitive manner. The inhibition constant (KI) has been found to be 6.6 x 10(-8) M. (Author abstract) (50 Refs)

9. THE ROLE OF CYTOPLASMIC MICROTUBULES IN THE REGULATION OF THE ACTIVITY OF PEPTIDE GROWTH FACTORS.

Friedkin M, Rozengurt E
Dept. Biology, Univ. California, San Diego, La Jolla, CA, 92093
Adv Enzyme Regul; 19:39-59 1981

The enhancement of DNA stimulation by peptide growth factors in colchicine-treated cultures of Swiss 3T3 mouse fibroblasts appears to be associated with disassembly of cytoplasmic microtubules. Other antitubulin agents (colcemid, vinblastine, podophyllotoxin, nocodazole, and maytansine) also potentiate the effects of growth promoting factors such as insulin and EGF. The cytoplasmic microtubules are disrupted by antitubulin agents at concentrations very similar to those required for depolymerization of mitotic spindle microtubules. In the presence of highly radioactive [3H]thymidine, DNA synthesis can be stimulated in quiescent mouse fibroblasts without completion of the cell cycle. This makes possible studies of the potentiating effects of colchicine during the prereplicative period of the cycle uncomplicated by effects of the antitubulin agent on the mitotic spindle. The enhancing effects of colchicine can be demonstrated with sparse cultures of 3T3 fibroblasts as well as with 3T6 cells. Maytansine and colchicine need not be present during the initial period of interaction of peptides with the cell to produce enhancement of DNA synthesis. The enhancement can be obtained after transient exposure of cells to fetal bovine serum. The potentiating effects of antitubulin agents such as nocodazole, podophyllotoxin, and maytansine can be easily reversed whereas the enhancement with colchicine persists despite washing of cells. The stimulatory effects of insulin and EGF persist in cultures treated with antitubulin agents. The findings support the premise that after internalization the receptor-mitogen complex may remain active within endocytic vesicles when cells are exposed to colchicine. (Author abstract) (71 Refs)

10. KINETIC, PHYSICAL, AND REGULATORY PROPERTIES OF AMIDOPHOSPHORIBOSYLTRANSFERASE.

Holmes EW
The Howard Hughes Medical Inst. Lab., Duke Univ. Medical Center, Durham, NC, 27710
Adv Enzyme Regul; 19:215-231 1981

Physical, kinetic, and regulatory properties of amidophosphoribosyltransferase (APRT), especially from mammalian sources, are reviewed. APRT catalyzes the synthesis of phosphoribosylamine (PRA), the first intermediate in purine ribonucleotide synthesis in mammalian cells. Studies have demonstrated two molecular forms of APRT. Interconversion between the two forms has a role in controlling the catalytic activity of APRT. Slow conversion of the large-inactive to the small-active form of APRT is associated with a lag phase before PRA synthesis reaches maximal velocity. Experiments indicate that human APRT is a hysteretic enzyme. Results suggest that APRT is an iron-sulfur protein; oxidation of the iron-sulfur center accounts for the oxygen-sensitivity of APRT. Activity of APRT corresponds to intracellular concentration of PP-ribose-P and availability of glutamine. Total APRT activity and presumably APRT concentration is increase in a number of malignant mammalian cell lines. The mechanism responsible for the increase in APRT synthesis or decrease in APRT degradation in malignant cells is not yet understood. Additional studies may identify factors which protect APRT from oxygen-inactivation and regulate APRT activity. (57 Refs)

11. MONO(ADP-RIBOSYL)ATION AND POLY(ADP-RIBOSYL)ATION OF PROTEINS IN DEVELOPING LIVER AND IN HEPATOMAS. RELATION OF CONJUGATE SUBFRACTIONS TO METABOLIC COMPETENCE AND PROLIFERATION RATES.

Bredehorst R, Wielckens K, Adamietz P, Steinhagen-Thiessen E, Hilz H
Physiologisch-Chemisches Institut, Universitat Hamburg, Universitats-Krankenhaus Eppendorf, Martinstrasse 52, D-2000 Hamburg 20, W. Germany
Eur J Biochem; 120(2):267-274 1981

Endogenous levels of mono(ADP-ribose)-protein conjugates are low in fetal liver. They increase during development reaching 30x higher levels in the adult stage. Undifferentiated hepatomas also exhibit low degrees of mono(ADP-ribosyl)ation compared with differentiated tumors. The observed changes cannot be explained by depolymerization of pre-existing protein-bound poly(ADP-ribose) groups or elongation of monomeric ADP-ribose residues since the monomeric and polymeric residues change independently, the absolute levels of residues present in the form of polymers being 20-350x lower than monomeric ADP-ribose residues. Subfractionation of the mono(ADP-ribose)-protein conjugates on the basis of their NH2OH sensitivity also showed independent changes during liver development. The level of the NH2OH-sensitive conjugates exhibit an inverse relationship to cell proliferation rates in normal and malignant hepatic tissues, while the NH2OH-resistant subfraction, which was hardly detectable in fetal liver, could be related to the degree of terminal differentiation (relative to adult liver). The ratio of NH2OH-resistant to NH2OH-sensitive mono(ADP-ribose)-protein conjugates being near unity in adult liver, fell to extremely low values in fetal and neonatal liver. In undifferentiated hepatomas (proliferating or stationary), however, the ratio was higher than in the adult normal tissue. This parameter, then allows one to discriminate between malignant and normal hepatic tissues with similar proliferative capacity and similar metabolic competence. On the basis of the findings presented it is suggested that covalent modification of proteins by mono(ADP-ribosyl)ation and poly(ADP-ribosyl)ation serve multiple and independent functions. (Author abstract) (62 Refs)

12. CHEMICAL STUDIES OF TISSUE POLYPEPTIDE ANTIGEN (TPA). III. ON THE NATURE OF THE ANTIGENIC DETERMINANT(S) OF TPA SUBFRACTION B1.

Wiklund B, Luning B, Bjorklund B
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Acta Chem Scand [B]; 35(5):325-336 1981

Tissue Polypeptide Antigen (TPA) which is a protein isolated from human carcinoma cells, has previously been separated into subfractions and studied with biochemical methods. Gel diffusion studies show that the antigenic determinants are retained through the isolation and purification procedures. Specific modifications of the amino acid residues lysine, tyrosine, tryptophan and arginine in subfraction B1 have been related to the change in the capacity of the antigen to bind to horse anti-HeLa serum. Complete although reversible loss of binding capacity resulted from blocking of arginine and a minor loss was noted upon modification of tyrosine. No measurable influence was noted upon modification of lysine or tryptophan. No cysteine has been detected in subfraction B1. Circular dichroism measurements show that TPA subfraction B1 is largely alphahelical in solution, and that no correlation could be detected between antigenic activity and conformation. (Author abstract) (33 Refs)

13. SPECIFIC, ANTIGENIC PROTEIN OF GUERIN EPITHELIOMA.

Palka JA, Rzeczycki W
Inst. Biochemistry and Medical Analytics, Medical Acad., 15-230 Bialystok 8, Poland
Neoplasma; 28(5):527-532 1981

Specific, antigenic protein of the mol wt 61,000 and 66,000 and isoelectric point 5.15 were isolated from cytosol of Guerin epithelioma and antiserum against these proteins was obtained. These antigens appear to be specific for epithelioma. Such antigens were not found in any normal rat organ. Blood serum of Guerin epithelioma bearing rats does not contain antibodies against these antigens. (Author abstract) (19 Refs)

14. BIOCHEMICAL AND IMMUNOLOGIC HETEROGENEITY OF IA GLYCOPROTEINS ISOLATED FROM A CHRONIC LYMPHOCYTIC LEUKEMIA.

Letarte M, Falk J
Div. Immunology, Hosp. Sick Children, 555 Univ. Ave., Toronto, Ontario M5G 1X8, Canada
J Immunol; 128(1):217-223 1982

Ia glycoproteins have been isolated from human chronic lymphocytic leukemic cells (CLL) by Lens culinaris chromatography and by filtration on ACA-34 Ultrogel. Ia antigenic activity, measured by inhibition of the cellular radioimmunoassay, was separated by gel filtration into two fractions, Peak I and Peak II. Monoclonal antibodies, produced against Peak II glycoproteins, appear to recognize different antigenic determinants of Ia molecules. Monoclonal antibody 18a4 reacted with Ia molecules of Peaks I and II, whereas monoclonal antibodies 18c2 and 18d5 reacted almost exclusively with Peak II molecules both in the cellular radioimmunoassay and by immunoprecipitation. In addition to antigenic differences, minor variations in the apparent mol wt of the Ia polypeptide chains were observed between Peaks I and II. These results indicate the existence of antigenically distinct subsets of Ia molecules that are separable by gel filtration. (Author abstract) (50 Refs)

15. NEURON SPECIFIC ENOLASE IN HUMAN SMALL CELL CARCINOMA CULTURES.

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Cancer Lett; 15(1):67-71 1982

Continuous cell lines of human lung cancer were analyzed for neuron specific enolase (NSE). The levels of NSE were determined in 21 cell culture samples derived from patients with small cell carcinoma of the lung (SCCL) and in 9 derived from patients with non-SCCL. The non-SCCL tumors were two mesotheliomas, three adenocarcinomas, two large cell carcinomas, a squamous cell carcinoma, and a mucoepidermoid tumor. NSE levels in the cells derived from small tumors are in every case except one higher than those derived from non-small cell tumors with the mean value of 997 nanog/mg protein. SCCL cells studied possess such amine precursor uptake and decarboxylation (APUD) cell characteristics as high levels of dopa decarboxylase and neurosecretory granules that they are distinct from other forms of lung cancer. NSE is strictly localized to neurons and neuroendocrine cells of the APUD series. NSE is present at much higher levels in the cultures derived from SCCL cells. The amounts of NSE present in the cultured SCCL cell lines ranged from 106-5071 nanog/mg protein. The highest value observed indicates that NSE represent 0.5% of the total soluble protein in the studied culture. Preliminary results concerning NSE levels in sera of patients with SCCL indicate that the levels of this protein may be useful in staging and following the course of the disease. (15 Refs)

16. BIOCHEMICAL COMPARISON OF MUTANT H-2KKVL(C3HFB/HEN) AND PARENT H-2KK(C3H/HEN) GLYCOPROTEINS.

Callahan GN, Walker LE, Martin WJ, Schirrmacher V, Bach FH, Nathenson SG, Rogers MJ, Demant P
Dept. Molecular Immunology, Scripps Clinic and Res. Foundation, 10666 North Torrey Pines Road, La Jolla, CA, 92037
Transplant Proc; 13(4):1787-1791 1981

H-2K antigens were isolated from C3H/HeN (C3H) and C3HfB/HeN (C3Hf) mice by immunoprecipitation using monoclonal antibodies against H-2Kk antigen. C3H and C3Hf splenocytes were labeled with [3H]arginine (3H-Arg) or [3H]lysine (3H-Lys). The heavy chains of the antigen were separated from beta-microglobulin, digested with trypsin, and analyzed by high-performance liquid chromatography. No apparent differences were found between tryptic peptides of H-2K antigens in 3H-Arg-labeled cells from C3H and C3Hf mice. Analysis of 3H-Lys-labeled cells showed two peptides in H-2K antigens from C3H mice that were absent from H-2K antigen from C3Hf mice. Analysis of peptides after treatment of the H-2K heavy chains with neuraminidase indicated that the H-2Kk molecule found in C3H mice differed from the H-2Kkvl molecule found in C3Hf mice in one or two peptide substitutions, rather than in altered glycosylation. Results suggest that a mutation has occurred in C3Hf mice at or near the K locus of the major histocompatibility complex. (8 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

17. CHARACTERIZATION OF THE MAJOR APOLIPOPROTEINS SECRETED BY TWO HUMAN HEPATOMA CELL LINES.

Zannis VI, Breslow JL, SanGiacomo TR, Aden DP, Knowles BB
Children's Hosp. Medical Center, Harvard Medical Sch., Boston, MA, 02115
Biochemistry; 20(25):7089-7096 1981

Two newly described human hepatoma derived cell lines, Hep G2 and Hep 3B, synthesize and secrete into the culture medium most of the major plasma apoproteins (apoA-I, apoA-II, apoB, apoC-II, apoC-III, ad apoE). The synthesized apoproteins were identified by direct two-dimensional gel analysis of the culture medium or by two-dimensional analysis following purification of the apoproteins by ultracentrifugation or immunoprecipitation. We found that the apoA-I synthesized by both of the hepatoma cell lines consists of two isoproteins designated 2 and 3 which are more basic than the major plasma apoA-I isoproteins designated 4 and 5. The apoE synthesized by both cell lines is composed mainly of an array of isoproteins with increasingly higher mol wt and lower isoelectric points as compared to those of the major apoE isoproteins found in plasma. These precursors of apoE are converted to the major apoE isoproteins upon treatment with Clostridium perfringens neuraminidase and represent sialo apoE isoproteins. ApoA-II, apoC-II, apoC-III-1, and apoC-III-2 correspond to the protein forms present in plasma. The human hepatoma cell lines (Hep G2 and Hep 3B) provide a unique model for studies of the regulation of human apoprotein and lipoprotein synthesis and catabolism. (Author abstract) (56 Refs)

18. STRUCTURAL STUDY OF THE SUGAR CHAINS OF ALPHA-AMYLASES PRODUCED ECTOPICALLY IN TUMORS.

Yamashita K, Tachibana Y, Takeuchi T, Kobata A
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J Biochem (Tokyo); 90(5):1281-1289 1981

About 30% of two alpha-amylases produced from a serous papillary cystadenocarcinoma of the ovarium (Case 1) and a bronchioloalveolar adenocarcinoma of the lung (Case 2) was glycoproteins containing 1 mol of asparagine-linked sugar chain, respectively. The structures of the sugar moieties were found by sequential enzymatic degradation and methylation analysis. Structures of asparagine-linked sugar chains were the same in the tumors of Cases 1 and 2 and were incomplete in comparison with those of the parotid amylase. (28 Refs)

19. IMMUNOLOGIC HETEROGENEITY OF THE FOLATE-BINDING PROTEINS FROM CHRONIC MYELOGENOUS LEUKEMIA CELLS AND MYELOFIBROSIS SPLEEN.

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Dept. Medicine, Brooklyn Veterans Admin. Medical Center, 800 Poly Place, Brooklyn, NY, 11209
J Lab Clin Med; 98(6):956-964 1981

An FBP has been purified from the spleen (FBP-S) of a patient with agnogenic myeloid metaplasia and myelofibrosis. This binder is similar to a previously purified protein from CML cells (FBP-L) in its mol wt and affinity for folate analogues. However, each protein has very little cross-reactivity in specific RIAs for each binder, indicating that there are structural differences which can be detected by the immune system. The concentration of FBP-L in the serum from normal subjects was 2.0 +-0.39 ng/ml (mean +- SEM) and was significantly elevated in the serum from some patients with CML, uremia, hepatitis, and cancer. The concentration of FBP-S, on the other hand, was 8.31 +-0.51 ng/ml in normal serum and remained in this range in the same serum that contained the elevated concentration of FBP-L. In contrast, the concentration of FBP-L was only 13% to 30% of the concentration of FBP-S measured in the homogenates of normal and adjacent cancerous tissue from lungs and colons. These studies indicate that some FBP(s) in human tissues have immunologic specificity even though they are functionally similar. (Author abstract) (25 Refs)

20. CATHEPSIN B AND ANTIPROTEASES IN PATIENTS WITH CARCINOMA OF THE CERVIX.

Benitez-Bribiesca L, Preyre-Horta R, Gallegos-Vargas G, De la Huerta-Sanchez R, Alfaro-Aceves L
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Arch Invest Med (Mex); 12(4):517-537 1981

Quantifiable cathepsin B elevations, their relation to the clinical development stage of neoplasia, and serum antiprotease concentrations were studied. One hundred eighty seven female patients divided into groups according to the stage of disease, were studied. Cathepsin B, alpha-1-antitrypsin (A1AT), alpha-2-macroglobulin (A2MG), trypsin inhibitory capacity, and total protein determinations were made in blood and vaginal fluid. Significant increase of cathepsin B in serum and vaginal fluid was detected in all patients (39/187) with carcinoma of the cervix in situ. Questionable elevation of thiol-proteinase was found in patients (33/187) with severe dysplasias. Cathepsin B elevations have a direct and linear correlation with the clinical stage of cancer reaching its highest levels in the most advanced stages. Serum A1AT is significantly increased from the clinical stage of carcinoma in situ and reaches its highest levels in the most advanced stages. In vaginal fluid A1AT concentrations increased significantly in patients stage Ib (7/187) and patients in more advanced stages. No changes were observed in A2MG concentration and in the inhibitory capacity of trypsin. Constant cathepsin B elevations in cancer patients are directly proportional to neoplasia development. A1AT levels also increased in blood. These elevations can be used as a diagnostic support in clinical diagnosis. (48 Refs)

21. ISOELECTROFOCUSING ANALYSIS OF ALPHA1-ACID-GLYCOPROTEIN (ALPHA1-AG) FOR PROGNOSIS OF GYNECOLOGICAL CANCER (MEETING ABSTRACT).

Kasuga K, Oaki A, Ohkawa R, Ohkawa K
Department of Obstetrics and Gynecology, Nippon Medical School, Tokyo, Japan
Obstetrics and Gynaecology: 8th Asian Congress, October 25-31, 1981, Melbourne, Australia. Asia and Oceania Federation of Obstetricians and Gynaecologists 352 pp., 1981.

The alpha 1-AG of cancer patients is considered to be an immunosuppressive agent. We tried to analyze the alpha-AG in blood of cancer patients by means of isolectrofocusing for early diagnosis and prediction of prognosis after treatment. The samples used for the experiment were the serum samples taken from patients with gynecological malignancy treated in this department. (Twenty cases of cervical cancer T1 15 of T2, 15 of T3-4, and 20 cases of recurrent cancer and 5 of hydatidiform mole). We used polyacrylamide gel plates of 1.5 mm thickness containing 1% of carrier ampholyt for the isoelectrofocusing. The samples were stained with Coomassie Brilliant Blue R-250. For the quantitative measurement the Single Radial Immuno-Diffusion method was used. The pattern of alpha1-AG separated by isoelectrofocusing was fixed in the agarose including alpha 1-AG-antiserum by means of two-dimensional electrophoresis. These patterns were nominated No1-No9 from the anode. In cases of good prognosis alpha 1-AG decreased 2 wk after treatment. In cases of poor prognosis alpha 1-AG did not decrease. In cases of ovarian cancer T2-T3 stage the level was 105+/-5.8mg/dl. In cases of cancer all patterns were increased, especially the 3th, 4th, 5th bands which were remarkably increased. In cases of good prognosis after treatment these patterns were decreased. In cases of healthy persons we did not observe the 1st and 9th bands. (no Refs)

22. THE SYNTHESIS OF A PROTEINASE INHIBITOR, ALPHA-1-ANTICHYMOTRYPSIN, BY HUMAN BREAST EPITHELIAL CELLS.

Tokes ZA, Gendler SJ, Dermer GB
Dept. Biochemistry, Sch. Medicine, Univ. Southern California, Los Angeles, CA, 90033
J Supramol Struct Cell Biochem; 17(1):69-77 1981

The biosynthesis and release of glycoproteins were studied as a function of breast epithelial cell arrangement in vitro. Benign and malignant human surgical specimens were maintained in organ culture under conditions where the three-dimensional tissue integrity was least altered. In order to confirm the synthesis of alpha-1-chymotrypsin (ACHY) in organ cultures and to estimate the proportion of labeled glycoproteins present as the proteinase inhibitor, quantitative immunoprecipitation was performed on culture supernatants. It was found that 3.5 and 7.8% of the total labeled glycoproteins from two benign breast tissues were recognized as ACHY by specific antibodies. Similarly, 0.9-5.4% of the total labeled components was precipitated from supernatants of three infiltrating ductal carcinomas. ACHY may represent a protective shield of cell surface components on breast epithelia, modulating the effects of invading leukocytes. (21 Refs)

23. A MULTIHORMONAL TUMOR OF THE PANCREAS PRODUCING NEUROTENSIN.

Feurle GE, Helmstaedter V, Tischbirek K, Carraway R, Forssmann WG, Grube D, Roher HD
Medizinische Poliklinik, Univ. Heidelberg, Hospitalstrasse 3, 6900 Heidelberg, W. Germany
Dig Dis Sci; 26(12):1125-1133 1981

In a pancreatic adenoma approximately 78.7% of the endocrine cells reacted specifically with antisera to neurotensin, 17.5% to gastrin, 2.8% to pancreatic polypeptide, and 1% to glucagon. The electron microscope revealed that the majority of the endocrine cells were N-cells -- morphologically similar to the ileal N-cells which are known to represent the neurotensin-producing cells. Neurotensin was extracted from the tumor and identified by Sephadex, ion-exchange, and high-pressure liquid chromatography. Gastrin, pancreatic polypeptide, and glucagon cells were also identified by the electron microscope; the peptides were extracted and demonstrated by chromatography. The serum concentrations of these hormones were elevated. After total gastrectomy which was necessary because of Zollinger-Ellison syndrome, a jejunoesophageal alkaline reflux, reaching the upper esophagus appeared. As intravenous infusion of synthetic neurotensin in rats caused an increase of luminal enteric pressure, it is suggested that severe jejunoesophageal reflux after gastrectomy may be a clinical feature of a neurotensinoma. (Author abstract) (24 Refs)

24. INTERACTION OF VASOACTIVE INTESTINAL PEPTIDE WITH HELA CELLS: ACTIVATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE AND LACK OF EFFECT ON DNA SYNTHESIS.

Prieto JC, Pintado E, De Miguel C, Guerrero JM, Goberna R
Catedra de Bioquimica, Facultad de Medicina, Alcala de Henares, Madrid, Spain
Biochem Biophys Res Commun; 103(3):799-805 1981

Protein kinase activity stimulated by vasoactive intestinal peptide (VIP) was studied in HeLa cells. Max activation required the simultaneous presence of a phosphodiesterase inhibitor. The response was dose dependent and in accordance with the concentration of VIP required for half-max stimulation of cyclic AMP production. Secretin stimulation of protein kinase and stimulation of DNA synthesis were also studied. (18 Refs)

25. CHARACTERIZATION OF MONONUCLEAR BLOOD CELL-DERIVED DIFFERENTIATION INDUCING FACTORS FOR THE HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE HL-60.

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JNCI; 67(6):1225-1230 1981

A search was made for physiologically produced differentiation inducing factor(s) (DIF) for the human promyelocytic leukemia cell line HL-60. Mononuclear blood cells, which stimulated with various mitogens, produced DIF, which induced differentiation of HL-60 into phagocytizing nitro blue tetrazolium reducing cells with the morphologic characteristics of granulopoietic or myelomonocytic cells. Induction of differentiation occurred equally well in serum-containing and serum-free media. Protein synthesis was necessary for elaboration of DIF, which seems to be of a protein nature inasmuch as it is destroyed by proteases. Gel chromatography showed that one or two species of DIF with apparent mol wt of 40,000 and 25,000 were produced, depending on the type of mitogen used. At least the 40,000-mol wt DIF was distinct from the colony stimulating activity (CSA), which was produced simultaneously. Our results suggested a role of lymphocytes and/or monocytes for modulation of myelomonocytic hematopoiesis not mediated by CSA. The physiologic importance remains, however, to be demonstrated. (Author abstract) (14 Refs)

26. EXAMINATION OF COLLAGEN STRUCTURE IN TISSUE OF WHITE RATS WITH SUBACUTE RADIATION SICKNESS.

Drozdz M, Antoniewica M, Kucharz E
Anstalt fur Klinische Chemie, Pharmakodynamisches Institut der Schlesischen Medizinischen Akadamie, Katowice, Poland
Z Gesamte Hyg; 27(10):753-755 1981

Collagen was isolated from tissues of rats exposed to sublethal doses (500 rads) of radiation. The amount of collagen in skin, liver, heart, lung, and kidney of irradiated rats was lower than that in corresponding tissues of control rats. The collagens from irradiated rats and from control rats also differed with respect to solubility and composition. (16 Refs) (In GER)

27. ANAPLASTIC HUMAN GLIOMAS GROWN IN ATHYMIC MICE: MORPHOLOGY AND GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION.

Jones TR, Bigner SH, Schold SC, Eng LF, Bigner DD
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Am J Pathol; 105(3):316-327 1981

The morphologic and biochemical characteristics of human surgical biopsy specimens taken from 17 patients with anaplastic human gliomas and of athymic mouse-grown tumors derived from them were examined. Fourteen were categorized as glioblastoma multiforme, one as an anaplastic astrocytoma, one as a recurrent glioblastoma multiforme, and one as a gliosarcoma. Fifteen of 17 tumors stained positively immunohistochemically for glial fibrillary acidic protein (GFAP), a glial-specific marker. When portions of the 17 surgical biopsy specimens were injected into the flank subcutaneous space of athymic mice, 16 produced tumors; different portions of a single biopsy specimen were used to establish three separate tumor lines; in toto, 18 tumor lines were established. Mouse-borne tumors contained various proportions of fibrillary and protoplasmic astrocytes, gemistocytes, small anaplastic cells, and multinucleated giant cells. Some were more homogeneous than the human tumors from which they were derived, while others contained a mixed population similar to that of the original biopsy specimen. Of these initial 18 tumors, 16 were stained for GFAP and 14 contained from fewer than 5% to almost 100% GFAP-expressing cells. Ten of the tumor lines were studied in serial passage, several demonstrating increased cellularity with increased passage. GFAP expression was followed through serial passage, and 7 of 10 tumor lines continued to express it, often in reduced amounts, and 2 of 10 ceased expression, one (the gliosarcoma) never having expressed it. These data demonstrate that while athymic mouse-borne human anaplastic gliomas retained some features of the human tumors from which they were derived, they varied from one another morphologically. These mouse-borne tumors also continued to evolve, often changing their levels of GFAP and demonstrating increased cellularity with passage. (Author abstract) (34 Refs)

28. VERNER-MORRISON SYNDROME.

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Ugeskr Laeger; 143(44):2881-2885 1981

The first case in Denmark of Verner-Morrison syndrome (VMS) in a 74-yr-old woman is reported, and the literature on VMS is reviewed. The syndrome was caused by a pancreatic tumor. Severe chronic diarrhea, hypokalemia and reduced gastric acid secretion were the cardinal symptoms. The vasoactive intestinal peptide (VIP) level in the serum, determined by radioimmunoassay, was increased significantly and correlated with the intensity of the disease. The tumor was detected by ultrasonic scanning and abdominal arteriography. Hypervascularized hepatic metastases were confirmed by biopsy. Long-term remission, cessation of diarrhea, and reduction of the VIP level to below 80 picomoles/liter were achieved by streptozotocin treatment, embolization of the hepatic artery, and interstitial irradiation. Recurrences were accompanied by rises in the VIP level. The review of the literature on VMS shows that VMS is frequently due to pancreatic VIP-secreting tumor, exclusively in adults, while neurogenic tumors (ganglioneuroma, neuroblastoma, ganglioneuroblastoma, pheochromocytoma, and neurofibroma) occur at all ages, most frequent in children. (30 Refs) (In DAN)

29. ANTIBODY DIVERSITY: SOMATIC HYPERMUTATION OF REARRANGED VH GENES.

Kim S, Davis M, Sinn E, Patten P, Hood L
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Cell; 27(3):573-581 1981

The immune response to phosphorylcholine in BALB/c mice has been well characterized. Amino acid sequence analyses of heavy-chain variable (VH) regions from 19 myeloma and hybridoma immunoglobulins binding phosphorylcholine show that 10 are identical (the prototype T15 VH sequence) and 9 are distinct variants differing by one to eight residues. A T15 VH DNA probe was used to isolate four closely related members of the T15 VH gene family, including one encoding the T15 VH sequence, from a sperm genomic library. A comparison of the protein and germline VH sequences suggested that most of the immune response to phosphorylcholine is derived from the T15 germline VH gene segment. The variant heavy chains from the M167 and M603 alpha immunoglobulins differ in their VH protein sequences from T15 by eight and three residues, respectively. We analyzed the somatic variability in and around the coding regions of these two variant VH genes by comparing them with the corresponding regions of the appropriate germline gene segments. The somatic variation has three properties: it is extensive and is found in flanking as well as coding sequences (for example, at least 44 substitutions for the M167 sequence and 10 substitutions for the M603 sequence); in the coding regions, it includes many silent as well as replacement substitutions; and it is focal in nature and centered around the rearranged VH genes. Although the mutations extend into the neighboring upstream and downstream flanking sequences, sequences approx 5 kb upstream and downstream from the VH genes show no substitutions. Moreover, the associated heavy-chain constant genes (Calpha) from both variant alpha genes are unaltered, indicating that a closely linked and coexpressed gene is unmutated. We conclude that this somatic variation is generated by a special hypermutational mechanism highly localized in its site of execution and highly restricted in its time of operation during B-cell development. (Author abstract) (43 Refs)

30. A GELATIN-BINDING 70,000-DALTON GLYCOPROTEIN SYNTHESIZED DISTINCTLY FROM FIBRONECTIN BY NORMAL AND MALIGNANT ADHERENT CELLS.

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J Biol Chem; 256(24):13085-13090 1981

Fibronectin is a high mol wt glycoprotein that has affinity for different types of collagens and especially for denatured collagen (gelatin). This property, characteristic of fibronectin, is widely used as a means of isolation of the protein. We now report on another gelatin-binding glycoprotein, Mr = 70,000, synthesized and secreted by various cultured adherent cells. The Mr = 70,000 polypeptide incorporates both radioactive amino acids and mannose, is monomeric, and contains intramolecular disulfide bridges as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing or reducing conditions. Several normal and malignant cell lines of both fibroblastic and epithelial type synthesize and secrete this polypeptide. Of the cell lines studied, only murine neuroblastoma C 1300 cells did not produce it although they produced fibronectin, while the Mr = 70,000 polypeptide is the only gelatin-binding protein produced by BeWo trophoblastic cells. No immunological cross-reactivity was detected between fibronectin and the Mr = 70,000 polypeptide as judged by electrophoretic protein transfer experiments and use of monoclonal anti-fibronectins and specific antisera against fibronectin and the Mr = 70,000 protein. Pulse-chase experiments in fibroblast cultures indicated that the Mr = 70,000 protein is synthesized separately from fibronectin and processed from an Mr = 68,000 precursor polypeptide. Isoelectric focusing showed that the Mr = 70,000 gelatin-binding proteins of different cell lines have identical pI values at 5.3-5.4. The Mr = 70,000 protein bound to gelatin-Sepharose as firmly as fibronectin, and both were eluted with 1 M arginine or 6 M urea but not with 3 M NaCL. The two proteins were separated by gel filtration, indicating that they are not associated with each other in fibroblast culture medium. Furthermore, the Mr = 70,000 protein produced by BeWo cells also binds to gelatin-Sepharose indicating that it binds independently of fibronectin. (Author abstract) (36 Refs)

31. 14C-ARGININE-RICH BASIC PROTEINS IN ASCITIC FLUID OF EHRLICH TUMOR.

Farbiszewski R, Zdrodowska J, Kilczewska D
Dept. Anorganic and Analytical Chemistry, Medical Sch., 15-230 Bialystok 8, Poland
Neoplasma; 28(5):617-619 1981

With the use of 14C-arginine it is shown that among the ascitic fluid proteins of Ehrlich tumor there appear basic proteins rich in arginine with the content of amino acid about 14%. The amount of these proteins is about 3%. They exhibit great similarity in respect to arginine content, electrophoretic mobility to the arginine rich basic proteins isolated previously from the cytosol of Ehrlich tumor cells. (Author abstract) (7 Refs)

32. RELATIONSHIP OF SERUM TOTAL CALCIUM TO ALBUMIN AND TOTAL PROTEIN IN DOGS.

Meuten DJ, Chew DJ, Capen CC, Kociba GJ
Dept. Veterinary Pathobiology, Ohio State Univ., Columbus, OH, 43210
J Am Vet Med Assoc; 180(1):63-67 1982

A positive linear relationship was found between total calcium and albumin and between total calcium and total protein in the serum of 209 dogs. Total calcium concentration correlated with the concentration of albumin (r = 0.575; P less than 0.001) and with the concentration of total protein (r = 0.411; P less than 0.001). A correction formula for calcium was derived on the basis of the concentration of albumin: adjusted calcium (mg/dl) = calcium (mg/dl) - albumin (g/dl) + 3.5. The correction formula for calcium, based on the concentration of serum total protein was adjusted calcium (mg/dl) = calcium (mg/dl) -0.4 [total serum protein (g/dl)] + 3.3. Hypocalcemia (less than or equal to 8.7 mg/dl) was detected in 32 of the dogs. After adjustment of the measured total calcium for albumin and serum total protein, 29 (91%) of the dogs had calcium concentrations within the normal range. Hypercalcemia was not associated with hyperalbuminemia or hyperproteinemia. In 91% of dogs with disorders of calcium metabolism and in 86% of dogs less than 6 mo old, calcium concentrations were outside the 95% confidence intervals for albumin and total protein calculated from the 209 dogs. It was concluded that adjustment of serum total calcium for protein concentration is essential for correct interpretation of calcium values and detection of abnormalities in calcium metabolism. (Author abstract) (16 Refs)

33. A LATENT THIOL PROTEINASE FROM ASCITIC FLUID OF PATIENTS WITH NEOPLASIA.

Mort JS, Leduc M, Recklies AD
Joint Diseases Lab., Shriners Hosp. for Crippled Children, 1529 Cedar Ave., Montreal, Quebec H3G 1A6, Canada
Biochim Biophys Acta; 662(2):173-180 1981

Pepsin treatment of ascitic fluid from patients with neoplasia generated a cysteine (thiol) proteinase activity which resembles cathepsin (EC 3.4.22.1) in its requirements for thiol activators, susceptibility to inhibitors and specificity for synthetic substrates. As judged by gel filtration, pepsin reduced the molecular size of the latent enzyme from an Mr of 41,000 to 33,000 after activation. Both forms are larger than human liver cathepsin B. In addition to its presence in ascitic fluid, the pepsin-activated species was found in the medium of ascites cells maintained in culture. The latent enzyme may be an enzyme-inhibitor complex or an inactive precursor of a cathepsin B-like proteinase. (Author abstract) (30 Refs)

34. HISTOCHEMICAL LOCALIZATION OF CATHEPSIN B AT THE INVASION FRONT OF THE RABBIT V2 CARCINOMA.

Graf M, Baici A, Strauli P
Div. Cancer Res., Inst. Pathology, Univ. Zurich, Zurich, Switzerland
Lab Invest; 45(6):587-596 1981

To clarify the role of cathepsin B in tumor invasion, the enzyme was visualized in tissue frozen sections of the subcutaneously growing rabbit V2 carcinoma. Localization of cathepsin B was achieved by immunofluorescent staining and by enzyme histochemistry. For the former approach, a sheep antiserum was raised against purified cathepsin B from rabbit liver. The antibodies, isolated by immunoadsorption, reaction monospecifically with rabbit liver cathepsin B in Ouchterlony double diffusion and in immunoelectrophoresis. In the enzyme histochemical assay, Z-Ala-Arg-Arg-methoxynaphthylamide was used as fluorogenic substrate and nitrosalicylaldehyde as coupling agent. With both methods, cathepsin B was found to be localized within fibroblasts and leukocytes assembled at the tumor invasion front. In addition, immunofluorescent staining demonstrated the occurrence of the enzyme in the extracellular matrix surrounding tumor cell clusters. Carcinoma cells always remained unstained. The conclusion is drawn that cathepsin B is chiefly produced by host cells which are stimulated to increase synthesis and to release the enzyme under the influence of the tumor. A dual function can be ascribed to cathepin B concentrated in the vicinity of the tumor: it operates intracellularly (in host cells) through degradation of endocytosed protein and extracellularly through activation of collagenase. The resulting lytic action on host structures appears to be a prerequisite for local spread of the V2 carcinoma. (Author abstract) (58 Refs)

35. THE COMPLETE V DOMAIN AMINO ACID SEQUENCES OF TWO MYELOMA INULIN-BINDING PROTEINS.

Johnson N, Slankard J, Paul L, Hood L
Dept. Biology, Stanford Univ., Stanford, CA, 94305
J Immunol; 128(1):302-307 1982

The myeloma proteins binding inulin afford a unique opportunity to study the V region patterns of variation because of the similarity in the VL as well as the VH regions. The diversity patterns in both the VL and VH regions suggest these proteins are encoded by multiple, very similar V gene segments or that somatic mutation may repeatedly generate identical variants. Because of the close similarity in the V domains of these proteins and the extensive idiotypic analyses that have been carried out previously, several interesting conclusions can be drawn about the nature of idiotypic determinants. First, a single amino acid residue may be involved in determining multiple idiotypic determinants. Second, hapten-inhibitable idiotypes may depend on residues within and outside the hypervariable regions. Third, idiotypic similarity does not always predict a corresponding sequence similarity. (Author abstract) (28 Refs)

36. BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF ECTOPIC TUMORAL RENIN.

Soubrier F, Devaux C, Galen FX, Skinner SL, Aurell M, Genest J, Menard J, Corvol P
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J Clin Endocrinol Metab; 54(1):139-144 1981

Biochemical and immunological characteristics of renin secreted by two malignant renin-secreting tumors [pulmonary (PT) and paraovarian (POT)] were studied. They both contain inactive renin (IR), as renin activity of tumoral extracts was able to be increased after acid activation or trypsin treatment (10.1 to 20.8 Goldblatt units/g tissue for PT and 1.4 to 3.71 for POT). Renin activity after activation reached the value obtained by direct RIA of human renin (23 and 3.4, respectively), as both forms are recognized by renin antiserum. Both enzymatic activities could be completely inhibited by renin antiserum. Displacement curves for the two tumoral renins paralleled the MRC renin in the direct RIA. After chromatography on affigel blue, active renin was not bound to the gel, and inactive renin eluted only with 1 M NaCl. On pepstatin A Sepharose and CBL-pepstatin Sepharose (an N-modified-pepstatin), a separation of the two forms of pulmonary renin was obtained; inactive renin eluted with breakthrough proteins, whereas active renin was strongly bound to the gel. After this affinity chromatography, the mol wt of inactive and active renin, determined on Ultrogel, were very close (46,000 and 42,500). We conclude that (1) ectopic renin in these cases is similar to the renal enzyme; (2) renin can be secreted in an inactive form, supporting the hypothesis of an inactive initial state of renin; and (3) mol wt differences between the two forms are very slight. (Author abstract) (25 Refs)

37. PURIFICATION AND CELL-SPREADING EFFECT OF GLYCOPROTEIN OF SPONTANEOUSLY MALIGNANT FIBROBLASTS.

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Dept. Pathology, Faculty Medicine, Kyoto Univ., Kyoto, Japan
Cell Mol Biol; 27(5):445-453 1981

Mouse fibroblast cells (m cells) and so-called reticulum cells (MR cells and SP cells), transformed into malignant cells in vitro, were transferred to a serum-free culture medium, and the morphological and functional differences of these two kinds of cells were studied. We found that the fibroblast cells produce a unique glycoprotein which has the biological function of inducing cell-spreading of so-called reticulum cells on the substratum in the serum-free culture medium. This glycoprotein was purified from the conditioned medium of the transformed fibroblast cells. The mol wt of this protein is about 230-250 x 10(3). The so-called reticulum cells themselves do not produce detectable amounts of this protein. Specific antiserum against this glycoprotein shows a single precipitation line against not only this glycoprotein, but also against serum protein. This antiserum inhibits the cell-spreading-inducing activity of this glycoprotein in vitro. Immunofluorescent microscopy shows that this glycoprotein exists on the cell surface and in the cytoplasm of normal and some transformed fibroblast cells. Undifferentiated fibrosarcoma cells, however, do not produce this glycoprotein in vitro. (Author abstract) (24 Refs)

38. THE USE OF THREE-DIMENSIONAL COLLAGEN GELS FOR THE STUDY OF TUMOUR CELL INVASION IN VITRO: EXPERIMENTAL PARAMETERS INFLUENCING CELL MIGRATION INTO THE GEL MATRIX.

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Int J Cancer; 29(1):57-62 1982

We have been using three-dimensional gels of native collagen fibers as a substratum for the study of cell migratory behavior. The objective of this work has been to develop an experimental model system for the study of tumor cell invasion. Data are presented in this communication concerning the effects of a number of experimental parameters on the migration of human foreskin fibroblasts (normal) and a Syrian hamster melanoma cell line (tumor) into three-dimensional gels of native collagen fibers. We find that the migration of both these cell types into the gel matrix is affected by the concentration of collagen in the gel, as well as by the initial density of cells plated onto the gel and the density of cells in the stock culture dishes. These results are discussed in terms of the control of cell migration into the gel and also demonstrate the potential utility of this model system for the study of tumor cell invasion. (Author abstract) (17 Refs)

BINDING/ RECEPTOR PROTEINS

39. RECOVERY OF BETA-ADRENERGIC RECEPTORS FOLLOWING LONG TERM EXPOSURE OF ASTROCYTOMA CELLS TO CATECHOLAMINE. ROLE OF PROTEIN SYNTHESIS.

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J Biol Chem; 256(23):12281-12286 1981

As a part of the process of agonist-induced desensitization, 1321N1 human astrocytoma cells lose up to 95% of their beta-adrenergic receptors, as detected by 125I-hydroxybenzylpindolol (125IHYP) binding, after 12-24 hr of exposure to isoproterenol. In preconfluent cultures the loss of beta-receptors is completely reversible upon removal of isoproterenol, with receptor levels reaching 100% of control levels within 48-72 hr. Addition of cycloheximide (5 ug/ml) upon removal of agonist does not prevent the recovery of receptors. After an initial 4-hr lag, receptors accumulate in the presence of cycloheximide until the same receptor level is reached that was present at the onset of desensitization. Confluent cultures, which have a reduced number of receptors per cell, recover beta-receptors to only 60 to 70% of control levels following removal of isoproterenol. In addition, cycloheximide blocks the recovery of receptors in these cultures. The effects of cycloheximide on the accumulation of receptors during cell growth suggest that receptors are stable in preconfluent cultures and that turnover only occurs later when cultures are confluent. The data also indicate that long term exposure of cells to catecholamine results in a form of the beta-adrenergic receptor that is undetectable by 125IHYP binding but, nonetheless, retains its primary amino acid structure. The undetectable receptors appear to be retained until agonist is removed, whereupon they become detectable by 125IHYP binding with a t 1/2 of about 36 hr in the presence of cycloheximide. (Author abstract) (32 Refs)

MEMBRANE/ SURFACE PROTEINS

40. ISOLATION AND CHARACTERIZATION OF C3B RECEPTOR-LIKE MOLECULE FROM MEMBRANES OF A HUMAN B LYMPHOBLASTOID CELL LINE (R

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FEBS Lett; 136(1):111-114 1981

A 140,000 mol wt glycoprotein carrying binding activity of the large fragment of trypsin-cleaved native third component of complement (C3b) was solubilized from the membrane of human B lymphoblastoid cell line, Raji. Some of the properties of this membrane component were analyzed. No change in mol wt of C3b-binding molecule was seen when sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis was performed in the presence of a reducing agent. Based on this results the presence of disulfide-linked subunits was ruled out. The presence of a carbohydrate moiety on this C3b-binding molecule was supported both by its radio-C labeling after intracellular labeling with 14C-glucosamine and by its properties of binding to Lens culinaris. No phosphorylation of the 140,000 mol wt C3b-binding molecule occurred after in vivo labeling of Raji cells by inorganic 32P and in vitro labeling of Raji membranes by gamma-32P-ATP before solubilization of membrane components. The ligand binding activity of this membrane component was tested taking advantage of its elution from C3b-Sepharose beads by acetic acid followed by immediate neutralization to pH 7.4; the acid-eluting C3b-binding protein was able to rebind to new C3b-Sepharose beads and could be eluted from the second affinity material by unlabeled C3b. It was suggested that partial denaturation occurred during the obligatory acid elution procedure and that the 140,000 mol wt glycoprotein extracted from Raji membrane and carrying a C3b binding affinity interacts as a C3b receptor and not as a C3d receptor. (20 Refs)

41. SURFACE GLYCOPROTEINS OF NORMAL AND MALIGNANT HEMATOPOIETIC CELLS.

Andersson LC
Pathology Inst., Helsinki Univ., 00290 Helsinki 29, Finland
Duodecim; 97(18):1509-1518 1981

Surface glycoprotein (GP) analysis was used to classify normal and malignant hematopoietic cells and to measure their degree of differentiation; the technique may be of importance in the prognosis and treatment of various types of leukemia. When GP120 becomes GP130 in the conversion of T-lymphocytes to killer cells, a definite change occurs in glycosylation, altering electrophoretic mobility. GP130 is specifically bound to the lectin of Vicia villosa, but the lectin does not react with GP120. When the promyelocyte grows into a granulocyte, the disappearance of GP160 and the appearance of GP130 is an example of altered glycosylation. Since GP160 and GP130 both react to the same antiserum, they clearly possess a common peptide component. Changes linked to differentiation during the glycosylation of surface glycoproteins can lead to the formation of differentiation antigens, important indicators of interaction between cells. A considerable part of the genetic information that measures surface changes in the cell during growth could be transmitted by way of various carbohydrate transferases. (22 Refs) (In FIN)

42. MEMBRANE PROTEIN REDISTRIBUTION DURING DIFFERENTIATION OF CULTURED HUMAN ERYTHROLEUKEMIC CELLS.

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Mol Cell Biol; 1(12):1150-1162 1981

Human erythroleukemic (K562) cells incubated with 0.05 milliM hemin undergo differentiation along the erythroid pathway. RBC proteins synthesized before and after such induction, morphology of the cells and localization of membrane proteins at the cell surface were examined. In the presence of hemin, cells continued to proliferate; after a 24-48 hr delay they started to synthesize Hb. During differentiation, no changes in major cell surface proteins were detected by lactoperoxidase-catalyzed iodination. Specific immune precipitation showed no change in synthesis of spectrin, the major cytoskeletal protein of the mature RBC. While no major changes in cell surface proteins were detected, scanning and transmission electron microscopy revealed marked changes in the morphologic organization of the cell membrane. A process similar but not identical to the enucleation observed in erythroid differentiation in vivo occurred: a smooth-surfaced cell approx 10 micron in diameter divided from the nucleus-containing part of the cell. Transmission electron microscopy showed that with the exception of ribosomes, these reticulocyte-like cells contained no organelles, but they contained much of the parent cell's Hb, spectrin, and glycophorin. (32 Refs)

43. SURFACE PROTEIN ABNORMALITIES IN LYMPHOCYTES AND PLATELETS FROM PATIENTS WITH WISKOTT-ALDRICH SYNDROME.

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Lancet; 2(8260):1387-1389 1981

Lymphocytes from three patients with the Wiskott-Aldrich syndrome were subjected to surface radioiodination and their 125I-labeled surface proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Autoradiographs demonstrated the absence in all three patients of an 125I-labeled protein, mol wt 115,000, that was present in normal individuals. In addition, one patient had an additional labeled protein, mol wt 135,000, not found in normal individuals. The platelets from one patient were radioiodinated and the 125I-labeled membrane proteins were analyzed. Glycoproteins Ia and Ib were reduced in amount and restricted in heterogeneity while glycoproteins IIb and IIIa were normal. These results suggest that the primary defect in the Wiskott-Aldrich syndrome may be abnormalities of (glyco)proteins normally present on the surface of lymphocytes and platelets. (Author abstract) (15 Refs)

44. IMMUNOLOGICAL DELINEATION IN NORMAL AND MALIGNANT CELLS OF A MEMBRANE PROTEIN INVOLVED IN GLUCOSE TRANSPORT. II. FUNCTION OF THE ANTIGEN.

Gingrich RD, Wouters M, Bramwell ME, Harris H
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J Cell Sci; 52:121-135 1981

A review of the literature suggested that the antigen detected by the M/27 antibody described in the preceding paper might be involved in the transport of glucose across the cell membrane. This idea was tested by measuring the amount of M/27 antibody bound to cells under a range of conditions known to influence the rate of glucose uptake. Glucose starvation and the administration of insulin under appropriate conditions are known to increase the Vmax for glucose uptake in certain cells, and high cell density in vitro is known to decrease the Vmax. Glucose starvation was found to increase the amount of M/27 antigen present on the surface of two malignant cell lines, but produced no detectable change in five different non-malignant cell types. Insulin increased the amount of M/27 antigen on the surface of both malignant and non-malignant cells. At high cell density, the amount of M/27 antigen present on the cell surface decreased. The changes in the Vmax for glucose uptake produced by these manipulations have been shown not to require the synthesis of protein. This was also found to be true for the changes in M/27 antigen concentration. By disrupting cells in detergent, it could be shown that there was an intracellular pool of M/27 antigen. The total amount of M/27 antigen in the cell was found to be about 4x as large as the amount of the antigen on the cell surface. This figure agrees with the value given in the literature for the size of the intracellular pool of the glucose transport system. The amount of M/27 antigen on the cell surface was found to vary during the cell cycle. A dramatic increase occurred early in S phase. (Author abstract) (31 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

45. HUMAN ADENOSINE DEAMINASE. PROPERTIES AND TURNOVER IN CULTURED T AND B LYMPHOBLASTS.

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J Biol Chem; 256(23):12496-12501 1981

Changes in the level of adenosine deaminase activity have been associated with normal T cell differentiation and T cell diseases, such as acute lymphoblastic leukemia; however, possible mechanisms controlling the level of this enzyme have not been explored. In this study, the properties and rate of turnover of adenosine deaminase are compared in cultured human T and B lymphoblast cell lines. Relative to B lymphoblasts, the level of adenosine deaminase activity in extracts of T lymphoblast cell lines is elevated 7-14-fold and differs by two-fold between the T cell lines. In both T and B lymphoblast extracts, the enzyme is apparently identical, based on Km for adenosine and deoxyadenosine, Ki for inosine, Vmax for adenosine, s20,w, isoelectric pH, and heat stability. Furthermore, by radioimmunoassay, the quantity of adenosine deaminase-immunoreactive protein is proportional to the level of enzyme activity in all cell lines studied. Using a purification and selective immunoprecipitation technique, the enzyme turnover could be assessed in cell lines labeled with [35S]methionine. The apparent rate of adenosine deaminase synthesis, relative to total protein, is two-fold faster in T cell lines than in the B cell lines studied. The apparent half-life (t1/2) for the enzyme degradation is 19-39 hr, respectively, in T cell lines while the t1/2 in both B cell lines is 7-9 hr. From the net rate of synthesis and degradation, the T cell lines, respectively, exhibit approximately a 6-and 12-fold difference in adenosine deaminase turnover relative to B cells, consistent with the observed differences in enzyme activity. This study suggests that while adenosine deaminase is apparently identical in both T and B lymphoblast cell lines, alterations in both the rate of enzyme synthesis and degradation contribute to its high steady state level in T cells. (38 Refs)

46. THE REGULATION OF PROTEIN SYNTHESIS IN HEART MUSCLE UNDER NORMAL CONDITIONS AND IN THE ADRIAMYCIN-CARDIOMYOPATHY.

Zahringer J
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Klin Wochenschr; 59(23):1273-1287 1981

The regulation of cardiac protein synthesis, in particular messenger-RNA (mRNA) and polyribosome metabolism, has been investigated in normal rat heart muscle and in the adriamycin-cardiomyopathy by using newly developed methods for the isolation, characterization and in vitro translation of cardiac polyribosomes and mRNA. (94 Refs)

47. EXPRESSION OF THE ALBUMIN GENE IN RAT HEPATOMA CELLS AND THEIR DEDIFFERENTIATED VARIANTS.

Cassio D, Weiss MC, Ott MO, Sala-Trepat JM, Fries J, Erdos T
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Cell; 27(2):351-358 1981

Rat hepatoma clones whose cells do and do not produce albumin, as well as somatic hybrids between the two types of cells, have been examined for albumin mRNA. A direct proportionality between the rate of albumin production and the concentration of albumin mRNA sequences was found for all albumin-producing hepatoma and hybrid clones, indicating that rate of synthesis of the protein is determined by the concentration of its mRNA. Albumin-negative dedifferentiated variant and somatic hybrid cells contain fewer than one to five molecules of albumin mRNA per cell; the block in expression of the gene appears to be at the same (probably transcriptional) level in variants and their somatic hybrids. (Author abstract) (50 Refs)

48. NEUROPHYSIN BIOSYNTHESIS IN VITRO IN OAT CELL CARCINOMA OF THE LUNG WITH ECTOPIC VASOPRESSIN PRODUCTION.

Yamaji T, Ishibashi M, Katayama S, Itabashi A, Ohsawa N, Kondo Y, Mizumoto Y, Kosaka K
Third Dept. Internal Medicine, Univ. Tokyo, Hongo, Tokyo 113, Japan
J Clin Invest; 68(6):1441-1449 1981

The incorporation of labeled compounds into neurophysins of a transplantable human oat cell carcinoma of the lung with ectopic vasopressin production was studied in vitro. Neurophysins in cell extracts and in incubation media were isolated by immunoprecipitation and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. When cells were incubated with L-[35S]cysteine for 12 hr, SDS-polyacrylamide gel electrophoresis of the immunoprecipitates from cell extract and medium resolved two forms of neurophysins with apparent mol mass of 10,000 (10K) and 20,000 (20K). Both forms of [35S]neurophysins were completely displaced from the immunoprecipitates by excess human neurophysin. Incubation of cells with L-[35S]cysteine and D-[3H]glucosamine hydrochloride revealed that glucosamine was incorporated into the 20K neurophysin region, but not into 10K species. To observe the kinetics of labeling of the two forms of neurophysins, cells were incubated with L[35S]cysteine for varying periods of time. After short labeling periods, most of the radioactivity resided in 20K species, which plateaued after 1 hr, whereas 10K neurophysin progressively increased in its height. When cells were chased with unlabeled cysteine after the exposure to a short pulse of labeling, 20K neurophysin peak gradually decreased with an apparent initial half-life of 1 hr. In contrast, the label in 10K neurophysin steadily increased, which exceeded the former by 3 hr of chase. Analysis of 20K neurophysin in cell extract by isoelectric focusing on polyacrylamide gel demonstrated that it was principally composed of a protein with an apparent isoelectric point (pI) of 5.7. These results suggest that neurophysin is synthesized in ectopic vasopressin-producing tumors by post-translational processing from a glycosylated proneurophysin with an apparent molecular mass of 20,000 daltons and a pI of 5.7. (Author abstract) (28 Refs)

49. DECREASED SYNTHESIS OF HIGH-MOLECULAR-WEIGHT GLYCOPEPTIDES IN HUMAN PROMYELOCYTIC LEUKEMIC CELLS (HL-60) DURING PHORBOL ESTER-INDUCED MACROPHAGE DIFFERENTIATION.

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Cancer Res; 42(2):484-489 1982

The human promyelocytic leukemia cell line, HL-60, synthesized a class of high-mol-wt (MW 5,000 to 7,000), N-linked glycopeptides as the major class of protein-bound carbohydrates. Small glycopeptides (MW 2,500 to 3,500), typical of most mammalian cells except RBC, represented a minor component in these cells. The large glycopeptides were labeled efficiently with fucose, glucosamine, and galactose but only poorly with mannose. They were found not to be glycolipids, glycosaminoglycans, or mucin-type glycopeptides and were not susceptible to exoglycosidases, but they were partially degraded by endo-beta-galactosidase. These characteristics are similar to those of the large glycopeptides synthesized by RBC, by another human myeloid leukemia cell line (K562), and by human and murine teratocarcinoma cells. High-mol-wt glycopeptides predominated on another human myeloid leukemia cell line KG1, but they were expressed at low levels on both a human monocytic leukemia cell line (THP-1) and a human T-lymphoblastoid cell line (Jurkat). when HL-60 cells were induced to differentiate into macrophage-like cells with phorbol esters, the proportion of large glycopeptides decreased, and the production of small glycopeptides predominated. This shift was observed within the first several hr after exposure to phorbol esters and was temporally related to the acquisition of adherent properties by the induced cells. In contrast, when HL-60 cells were induced to differentiate into granulocytes by dimethyl sulfoxide, hypoxanthine, or retinoic acid, they continued to synthesize glycopeptides similar to uninduced cells. Human peripheral blood granulocytes synthesized primarily large glycopeptides, whereas monocytes and lymphocytes synthesized mostly small glycopeptides. These results indicate that the synthesis of high-mol-wt glycopeptides is a property of human myeloid leukemia cell lines and that it persists throughout myeloid differentiation. A proportionate decrease in the synthesis of these large glycopeptides is a part of the differentiation program for monocytes and macrophages. (Author abstract) (34 Refs)

50. AUTORADIOGRAPHIC STUDY ON THE ROLE OF DIFFERENT RIBOSOME TYPES IN HUMAN LYMPHOBLASTS TREATED WITH VINBLASTINE.

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Cell Mol Biol; 27(5):483-489 1981

Human lymphoid cells have been treated in vitro with high doses of vinblastine sulfate which induce the formation of microtubule crystals surrounded by clusters of ribosomes and of isolated rows of granulofilamentous material associated with long regular polysome helices. The role of these ribosome associations, previously described in other cell types, has not been clarified. A high-resolution autoradiographic analysis has been made in order to determine whether these two types of ribosome associations are engaged in protein synthesis. The results indicate that the ribosomes associated with the microtubule crystals are active in protein synthesis and that the neosynthesized proteins are precipitated by the vinblastine in a crystalline form, while the regular polysomes are completely inactive since they are probably neosynthesized units transported along the cytoskeleton components. (Author abstract) (25 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

51. EFFECT OF 12-O-TETRADECANOYL-PHORBOL-13-ACETATE ON CA2+ EFFLUX AND PROTEIN DISCHARGE IN PANCREATIC ACINI.

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J Biol Chem; 256(23):12040-12045 1981

When pancreatic acini are exposed to the peptide cerulein (an analogue of the hormone cholecystokinin) or the cholinergic agonist carbamylcholine, they exhibit a rapid release of intracellular Ca+ + and a rise in the level of cGMP, accompanied by discharge of secretory proteins. The phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) stimulates protein discharge without affecting the level of cGMP, while N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) elevates cGMP, but does not elicit discharge. To clarify the relationships among these events, the effect of TPA and MNNG on Ca+ + efflux in guinea pig acini was determined. Cells were incubated in medium containing 45Ca+ + and transferred to medium with nonradioactive Ca+ + before adding the agents to be tested. Cerulein (10(-9) M), carbamylcholine (10(-5) M), and the Ca+ + ionophore A23187 (5 ug/ml) all caused the release of 40-60% of the cell-associated 45Ca+ + within 3 min. In contrast, acini incubated with 10(-7) M TPA, 10(-4) M MNNG, or no added agents retained over 80% of their 45Ca+ + after 22 min. TPA caused significant secretory discharge within the first 15 min and did not block Ca+ + efflux stimulated by other secretagogues. TPA-induced discharge requires energy and the presence of sufficient Ca+ + in the extracellular medium. These observations are consistent with a model in which the rapid release of Ca+ + into the cytoplasm triggers an increase in the level of cGMP and, by a separate series of events, protein discharge. TPA may act at a later point on the pathway to discharge, but before an obligatory step requiring Ca+ +. (Author abstract) (31 Refs)

52. STIMULATION OF TRANSFER OF METHYL GROUPS FROM O6-METHYLGUANINE IN DNA TO PROTEIN BY RAT LIVER EXTRACTS IN RESPONSE TO HEPATOTOXINS.

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Carcinogenesis; 2(11):1195-1200 1981

An enzymatic activity present in rat liver extracts catalyzes the transfer of me thyl groups from O6-methylguanine in DNA to protein. This activity was stimulated by treatment of rats with thioacetamide, carbon tetrachloride, 1,2-dimethylhydrazine, diethylnitrosamine, dimethylnitrosamine and by partial hepatectomy but not by treatment with N-methyl-N-nitrosourea or streptozotocin. These results suggest that an enhancement of this activity accompanies the increase in cell division brought about by these agents and is not necessarily a specific response to the presence of alkylated bases in DNA. (Author abstract) (38 Refs)

53. INCREASES IN PROTEIN A24 FOLLOWING TREATMENT OF EHRLICH ASCITES TUMOR CELLS WITH 1-METHYL-1-NITROSOUREA AND 1,3-BIS(2-CHLOROETHYL)-1-NITROSOUREA.

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FEBS Lett; 136(1):41-44 1981

Following treatment of Ehrlich ascites cells with 1-methyl-1-nitrosourea (MNU) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), densitometric analysis of one-dimensional gels revealed that protein A24 (a conjugate of histone H2A a ubiquitin) increased in a dose-dependent manner, while protein synthesis was totally inhibited. Treatment of cells with 50 ug/ml BCNU led to 247x more A24 than in control cells. Treatment with 200 ug/ml MNU led to 307x more A24 than in controls. Increases of protein A24 were also demonstrated by two-dimensional gel electrophoresis. The simultaneous presence of puromycin (20 ug/ml) had no effect of protein A24, indicating that protein synthesis was not responsible for the larger amounts of A24 seen after nitrosourea treatment. Possible ways in which nitrosoureas could cause the apparent increases in protein A24 included alkylation by BCNU and MNU changing the structure of protein A24 and preventing its cleavage mediated by A24 lyase, preventing A24 cleavage through DNA crosslinking, or inhibition of A24 lyase nitrosourea-treated cells. (23 Refs)

54. ANALYSIS OF INTERFERON MRNA IN HUMAN FIBROBLAST CELLS INDUCED TO PRODUCE INTERFERON.

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Proc Natl Acad Sci USA; 78(12):7426-7430 1981

The levels of interferon mRNA as a function of interferon induction by poly(rI)-poly(rC) in human fibroblast cells were determined by RNA hybridization using a cloned beta interferon cDNA and by translation in Xenopus oocytes. Whereas previous studies analyzed mixtures of interferons, the availability of the cloned beta interferon cDNA and the antiserum to purified beta interferon enabled us to focus on the expression of only one class (beta) of interferon genes. The induction of interferon synthesis depends primarily on the accumulation of interferon beta mRNA in the cells, and the interferon beta mRNA rapidly disappears several hours after its appearance in the cytoplasm. No detectable interferon beta mRNA sequences are present in uninduced cells. The degradation of interferon beta mRNA in the induced cells requires ongoing protein synthesis; accumulation of interferon beta mRNA was observed in the continuous presence of cycloheximide. The interferon beta mRNA detected at the early stages of induction is 1,100 nucleotides long and its size progressively decreases with time. By both the hybridization and the translational assay in Xenopus oocytes, only one size of interferon beta mRNA and one species of beta interferon could be identified. (Author abstract) (33 Refs)

55. INHIBITION OF COLLAGEN ACCUMULATION BY GLUCOCORTICOIDS IN RAT LUNG AFTER INTRATRACHEAL BLEOMYCIN INSTILLATION.

Sterling KM, DiPetrillo T, Cutroneo KR, Prestayko A
Coll. Medicine, Univ. Vermont, Burlington, VT, 05405
Cancer Res; 42(2):405-408 1982

Male Fischer 344 rats were given a single lung instillation of bleomycin sulfate (0.6 units/100 g). Some animals were treated 24 hr after bleomycin administration with triamcinolone diacetate. Steroid treatment was continued on alternate days for 4 weeks. At the end of 4 weeks, the lungs of rats receiving bleomycin alone had two-fold increases of both prolyl hydroxylase activity and proteinaceous hydroxyproline as compared to control values. The lungs of bleomycin-treated rats which received 4 mg of triamcinolone diacetate per kg on alternate days had a 33% increase of prolyl hydroxylase activity and a 37% increase of proteinaceous hydroxyproline content as compared to control values. Lung prolyl hydroxylase activity and proteinaceous hydroxyproline content of bleomycin-treated animals receiving glucocorticoid (8 mg/kg) on alternate days were the same as control values. The results indicate that alternate day administration of the synthetic glucocorticoid triamcinolone diacetate blocks lung collagen accumulation following a single intratracheal dose of bleomycin to rats. (Author abstract) (38 Refs)

56. THE ROLE OF PROTEIN ACCUMULATION IN THE CELL CYCLE CONTROL OF HUMAN NHIK 3025 CELLS.

Ronning OW, Lindmo T, Pettersen EO, Seglen PO
Dept. Tissue Culture, Norsk Hydro's Inst. for Cancer Res., Norwegian Radium Hosp., Montebello, N-Oslo 3, Norway
J Cell Physiol; 109(3):411-418 1981

Cycloheximide (CH) at concentrations of 0.125-1.25 uM partially inhibited protein synthesis, and slowed down traverse through the cell cycle. Median cell cycle duration equaled protein doubling time in both normal and CH-treated cells, possibly indicating that cell division cannot occur until a critical cells size or protein accumulation-dependent sequence of events has occurred. G1 duration increased in response to CH, but entry of cells into S, once initiated, was virtually unaffected. DNA synthesis in CH-treated cells began when protein content was lower than in control cells. (27 Refs)

DIETARY AND NUTRITIONAL EFFECTS

57. CHANGES IN NITROGEN METABOLISM IN CATABOLIC PATIENTS GIVEN THREE DIFFERENT PARENTERAL NUTRITION REGIMENS.

Tulikoura I, Huikuri K
Second Dept. Surgery, Helsinki Univ. Central Hosp., Helsinki, Finland
Acta Chir Scand; 147(7):519-524 1981

Nitrogen metabolism was investigated in 24 catabolic patients (15 men and 9 women) given three different parenteral nutrition regimens for 10-12 days. Energy was supplied as either carbohydrates alone (glucose group) or as carbohydrates and fats (lipid group) and nitrogen as amino acids. In the third group (amino acid group) amino acids were given in excess and less energy was supplied as carbohydrate. Each patient served as his own control. On the basis of daily urinary urea excretion all infused amino acid was retained in the body in the glucose and lipid groups during parenteral nutrition, but in the amino acid group 54% of infused amino acids were metabolized in gluconeogenesis apparently for energy production. In the glucose and lipid groups nitrogen balance rose to a 'plateau' within 2-4 days but in the amino acid group within 5-7 days. The increment of nitrogen balance, 0.169 +-0.030 g N/kg/day, in the glucose group was greater than that, 0.140 +-0.037 g N/kg/day, in the lipid group (p less than 0.05) and that, 0.122 +-0.044 g N/kg/day, in the amino acid group (p less than 0.01). The initially low values of serum and liver protein did not change in the glucose and lipid groups, but in the amino acid group serum protein rose from 53.7 +-6.5 g/l to 61.1 +-5.9 g/l (less than 0.01) and liver protein from 47.4 +-7.2 mg/mg DNA to 65.0 +-22.6 mg/mg DNA (p less than 0.05). It seems that a parenteral nutrition program rich in amino acids stimulates the vital protein synthesis of the liver more than regimens with abundance of non-nitrogen energy sources and poorer in amino acids. Nitrogen balance does not reveal information about the important protein metabolism of the liver. The results of this study support the opinion that during parenteral nutrition carbohydrates improve nitrogen balance more than isocaloric amount of fat. (Author abstract) (29 Refs)

58. EFFECT OF PARENTERAL NUTRITION ON IMMUNE STATUS OF PATIENTS SUBJECTED TO SURGERY FOR CANCER OF THE STOMACH.

Rybkin MP, Salmanova EA, Frid IA, Bernshtein MI
Dept. Anesthesiology and Resuscitation, N. N. Petrov Res. Inst. Oncology, Leningrad, USSR
Vestn Khir; 127(10):24-28 1981

The effect of parenteral nutrition on immune status was studied in 48 patients (30 men, 18 women; 30-70 yr old) subjected to subtotal stomach resection for cancer of the stomach.

Within 3-5 days after the surgery, 24 patients (Group I) received complete parenteral nutrition with amino acid mixtures, lipid emulsions, glucose, and electrolyte soln, while 24 other patients (Group II) received conventional infusions of glucose and electrolyte soln. The complete parenteral nutrition did not affect the number of T-lymphocytes. The patients in Group I showed increases in levels of IgG and IgM, while the patients in Group II showed decreases in these levels throughout the entire postoperative period. Postoperative complications (pneumonia, abscess, phlegmon) were observed in 4/24 patients in Group I and in 6/24 in Group II. (3 Refs) (In RUS)

59. PATHOPHYSIOLOGY OF CANCER CACHEXIA: CURRENT UNDERSTANDING AND AREAS FOR FUTURE RESEARCH.

DeWys WD
Nutrition Section, Clinical Investigations Branch, Div. Cancer Treatment, NCI, Bethesda, MD, 20205
Cancer Res; 42(2,Suppl):721S-726S 1982

Weight loss and failure to gain weight normally in cancer patients are attributable to negative energy balance and altered metabolism. Energy balance is negative because of decreased intake, increased expenditure, or both. Changes in carbohydrate metabolism include glucose uptake and lactate production by tumor, relative hypoinsulinism, and relative insulin resistance. Alterations in protein metabolism include preferential uptake of amino acids by the tumor, decreased synthesis of some host tissue proteins such as muscle tissue, and increased synthesis of other host proteins. Lipid metabolism is seemingly less affected. These metabolic changes result in muscle wasting in adult cancer patients and growth failure in pediatric cancer patients. Host tissues are catabolized to meet the nutritional demands of the tumor, and nutritional death may ensue. (Author abstract) (34 Refs)

60. TUMOUR INDUCTION OF HOST LEUCINE STARVATION.

Lazo PA
Dept. Biochemistry, Univ. Cambridge, Tennis Court Road, Cambridge, CB2 1QW, England
FEBS Lett; 135(2):229-231 1981

Ascites carcinoma cell requirements for energy and leucine are reported and compared with daily exogenous requirements of the host. Aerobic glycolysis and glutamine oxidation provide 80% and 20% of the total energy production of the tumor, respectively. Lactate reconversion to glucose by the host liver or peripheral lactate oxidation represent less than 0.5% and less than 1% increases in daily host energy requirements, respectively. Apparently both processes occur in the host. Tumor size is positively correlated with leucine intake. The rate of leucine use by the tumor is 30 nanomoles/min/g cells, making the tumor a drain on leucine sources. Characteristics of different amino acid pools and interaction of these pools in the organism can account for the selective and rapid use of nutrients by tumors. Tumor drain on leucine reserves may account for cachexia, the main cause of death in tumor patients. (16 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

61. URINARY POLYAMINE EXCRETION DURING GROWTH AND REGRESSION OF 7,12-DIMETHYLBENZ[A]ANTHRACENE-INDUCED RAT MAMMARY CARCINOMA.

Anehus S, Albinsson A, Andersson G, Heby O
Dept. Zoophysiology, Univ. Lund, Helgonavagen 3b, S-223 62 Lund, Sweden
JNCI; 67(6):1265-1268 1981

The correlation of urinary excretion of polyamines and tumor mass was examined with the use of a controlled experimental model. Mammary carcinoma growth was induced in Sprague-Dawley virgin rats by intragastric administration of 7,12-dimethylbenz[a]anthracene. Tumors were palpable after about 45 days. After a period of growth, regression of the estrogen-dependent tumors was induced by bilateral ovariectomy. Tumor volume and 24-hr urinary excretion of polyamines were measured during the course of tumor growth and regression. Urinary polyamines were analyzed after acid hydrolysis to determine the total amount of bound and free polyamines. Putrescine excretion followed closely the changes in tumor volume during the course of tumor growth and regression. Urinary spermidine excretion, however, remained essentially unchanged in ovariectomized rats; spermine was barely detectable in any of the urines. There was a high positive correlation between the 24-hr urinary putrescine excretion and urine volume. In nonovariectomized rats, the mammary tumor(s) continued to grow. An unexpected result of the advanced tumor progression was that urinary excretion of both putrescine and spermidine decreased steadily with time as did urine volume. This phenomenon may be due to the fact that tumor-bearing rats develop a state of overt malnutrition, involving complex disturbances of the host metabolism, manifested in decreasing body wt. (Author abstract) (15 Refs)

62. POLYAMINE LEVELS IN CSF FROM PATIENTS WITH PITUITARY TUMORS OR NONNEOPLASTIC PITUITARY DISEASE.

Fulton DS, Marton LJ, Lubich WP, Wilson CB
(c/o Marton) Brain Tumor Res. Center, Dept. Neurological Surgery,, HSW 783 Univ. California, San Francisco, CA, 94143
Arch Neurol; 39(1):47-48 1982

The relationship of cerebrospinal fluid (CSF) polyamine levels to tumor type and size was studied in patients with pituitary adenoma, cyst, abscess, or empty sella syndrome, and in a reference group composed of 5 healthy adults and 25 patients with non-neoplastic neurologic diseases. The mean putrescine level in CSF of 21 patients with pituitary adenomas (247 picomoles/ml) was significantly higher than that in CSF of the reference group (184 picomoles/ml), but spermidine levels were not significantly different in the two groups (193 and 142 picomoles/ml, respectively). No significant difference was observed in either putrescine or spermidine levels in CSF of patients with suprasellar extension of the tumor compared with those with no suprasellar extension. Putrescine and spermidine levels were significantly elevated in CSF of 1/4 patients with empty-sella syndrome and in one patient with a pituitary abscess, but fell within the range of the reference group for a patient with a pituitary cyst and for the other three patients with empty-sella syndrome. These results suggest that CSF polyamine levels have no significant diagnostic role in patients with pituitary disease. (16 Refs)

63. UPTAKE OF PUTRESCINE BY 3T3 AND SV3T3 CELLS AND ITS EFFECT ON ORNITHINE DECARBOXYLASE ACTIVITY.

Bethell DR, Pegg AE
Dept. Physiology, Milton S. Hershey Medical Center, Hershey, PA, 17033
J Cell Physiol; 109(3):461-468 1981

Addition of exogenous putrescine (PS) to cells during serum stimulation resulted in marked increase in intracellular PS and prevented rise in ornithine decarboxylase. PS transport of putrescine was inhibited by cycloheximide. The putrescine uptake system is apparently different from the amino acid uptake system. Cadaverine (1,5-diaminopentane) was accumulated much more slowly than putrescine, and uptake was not saturable under any of the conditions tested. (34 Refs)

OTHER RELATED STUDIES

64. DIFFERENCES IN CYTOSOL FACTORS IN LIVER AND HEPATOMAS REVEALED BY REAL OR APPARENT EFFECTS ON THE INCORPORATION OF [3H]THYMIDINE.

Barra R, Collin GR, Parsons J, Lea MA
Dept. Chemistry and Chemical Engineering, Stevens Inst. Technology, Hoboken, NJ, 07030
Int J Biochem; 13(12):1233-1240 1981

Effects of ethanol-extracted cytosol factors from rat liver and from Morris hepatomas on precursor incorporation was studied in hepatocytes and hepatoma cells. While precursor incorporation occurred, a portion of the effects may have been due to artifacts in the extraction procedure. Influence of cytosol fractions on incorporation of 3H-labeled thymidine and amino acids into nucleic acids and proteins was studied in hepatocytes from rats and HTC cells derived from the Morris hepatoma 7288C. Quantitative differences between tissues were seen most readily after precipitation of cytosol fractions with 45-70% ethanol. Effects were greater when cytosol fractions from normal or regenerating liver were used and incubations were made with hepatoma cells. An inhibitory action on the incorporation of (3H)thymidine into DNA, accompanied by increased incorporation into a residual protein fraction, was partially attributable to a requirement for more drastic conditions for DNA extraction in the presence of the cytosol fraction. Studies with (3H)lysine and (3H)leucine also resulted in increased incorporation into protein while long-term studies resulted in inhibition of cell proliferation. Results indicate a type of artifact not generally considered in the study of factors affecting (3H)thymidine incorporation. The data indicate changes in the cytosol which are a feature of neoplastic but not normal liver growth. (13 Refs)

65. MECHANISM OF GROWTH STIMULATION OF L1210 CELLS BY 2-MERCAPTOETHANOL IN VITRO. ROLE OF THE MIXED DISULFIDE OF 2-MERCAPTOETHANOL AND CYSTEINE.

Ishii T, Bannai S, Sugita Y
Div. Biochemistry, Tsukuba Univ. Sch. Medicine, Sakura, Niihari, Ibaraki, 305, Japan
J Biol Chem; 256(23):12387-12392 1981

The mechanism of the growth-promoting action of 2-mercaptoethanol on mouse lymphoma L1210 cells in vitro has been studied. The normal line of L1210 is deficient in its capacity to take up cystine, and this limits the growth of the cells in normal culture media containing cystine. In the presence of 2-mercaptoethanol, the cells obtain a high capacity to utilize cystine in the medium. The reaction of 2-mercaptoethanol with cystine produces a mixed disulfide of 2-mercaptoethanol and cysteine. The mixed disulfide is taken up by the cells mainly via the L system, a transport system for neutral alpha-amino acids such as leucine. The mixed disulfide within the cells is rapidly reduced to produce cysteine and 2-mercaptoethanol which escapes rapidly into the medium and reacts with cystine again. With the aid of this cyclic action of 2-mercaptoethanol, the cells are able to utilize cystine constantly. (Author abstract) (29 Refs)

66. IMMUNOLOGICAL CONTROL OF TUMOR GROWTH THROUGH CHEMICALLY PRETREATED BIOMPHALARIA SNAILS EXTRACTS.

Nabih I
Natl. Res. Centre, Dokki, Cairo, Egypt
Cell Mol Biol; 27(2/3):283-285 1981

Subcutaneous injection of nucleoprotein extracts from chemically pretreated Biomphalaria snails, the intermediate host of Schistosoma mansoni, caused regression in both size and cell count in different types of experimental tumors. Full cure was commonly observed in mice leukemia of EL4 type. (Author abstract) (8 Refs)

67. MEMBRANE PROTEINS IN HUMAN SKIN TUMORS.

Landi FL, Cardillo A, Fabrizi G, Farallo A, Miani N, Serri F
(c/o Serri) Dept. Dermatology, Catholic Univ., Largo A. Gemelli 8, I-00168 Rome, Italy
Arch Dermatol Res; 271(4):419-427 1981

The microsomal, mitochondrial, and nuclear fractions of 50 cutaneous tumors, chromatographed on sodium dodecyl sulfate-acrylamide slab gels, revealed electrophoretic profiles (EP) of microsomal proteins which were characteristic of some tumors. Banding patterns of membrane proteins from tumor cells indicated molecular weights around 11,700 daltons and 25,000-40,000 daltons. While normal skin showed relatively simple and reproducible EP of microsomal proteins, those of skin tumors contained more bands of proteins, with qualitative and quantitative differences. Squamous cell and intermediate epitheliomas showed similar EP, which may indicate a relationship between the two cancers. Banding patterns of melanotic melanomas tended to form clusters similar to squamous cell epithelioma, but to a lesser extent. The EP of amelanotic melanoma was different from that of melanotic melanomas. The EP of fibrohistiocytoma showed some similarities to amelanotic melanoma. Mitochondrial membrane proteins of squamous cell and intermediate epitheliomas and normal skin were similar. Melanotic melanoma showed reproducible EP but with more bands in the range greater than 60,000 daltons than in squamous cell epithelioma and normal skin. The EP of nuclear membrane proteins of all squamous cell epitheliomas were reproducible, but with some qualitative and quantitative variations; EP of intermediate epitheliomas were similar. (9 Refs)

68. HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN RAT KIDNEY AFTER CRYOSURGERY.

Sindelar WF, Javadpour N, Bagley DH
Surgery Branch, NCI, NIH, Building 10, Room 10N-116, Bethesda, MD, 20205
J Surg Oncol; 18(4):363-379 1981

Effect of cryoinjury to normal rat kidney was studied at the light and electron microscope level, and characterization of the time course of pathologic changes in kidney tissue after cryosurgery was described from 1 hr up to 14 days after production of a standard cryolesion. Cryoinjury to the kidney resulted in sharply demarcated lesions with minimal inflammatory response during the period of cell destruction and healing by fibrosis. Cellular protein was completely coagulated within 1 hr after cryoinjury, with crystallization of protein and disruption of membranes. Cellular disruption was rapid, and the clearance of cellular debris from the area of injury by macrophage infiltration was accomplished through a quite mild inflammatory reaction. Supporting stromal elements of renal parenchyma remained intact, including collagen tubular framework and basement membranes, and formed the lattice over which fibrosis developed. (Author abstract) (9 Refs)

69. EFFECT OF H+ ON THE KINETICS OF NA(+)-DEPENDENT AMINO ACID TRANSPORT IN EHRLICH ASCITES TUMOR CELLS: EVIDENCE FOR H+ AS AN ALTERNATIVE SUBSTRATE.

Smith TC, Robinson SC
Dept. Physiology, Univ. Texas Health Science Center, San Antonio, TX, 78284
J Cell Physiol; 109(3):507-516 1981

The kinetic response of Na+-dependent alpha-aminoisobutyric acid (AIB) transport to H+ was investigated in Ehrlich ascites tumor cells. Initial AIB fluxes were measured in the presence of varying external Na+ and H+ concentrations. Elevation of H+ at both high (154 mEq/liter) and low (10 mEq/liter) of external Na+ concentrations decreased max influx and increased the apparent Km for AIB influx. In the presence of very low concentrations of Na+ (0.94 mEq/liter), alterations in H+ had no effect on AIB flux. Two kinetic models are proposed which are consistent with the experimental results: (1) an order bireactant (Na+ binding necessary before AIB binding) system or (2) a random bireactant system. Both models require H+ to serve as an alternative substrate for Na+. Test of fit of both models to data from the present study gave similar results. (30 Refs)

70. ENHANCED AMINO ACID TRANSPORT IN CULTURED HEPATOCYTES DURING LIVER DEVELOPMENT.

Bellemann P
Dept. Pharmacology, P.O. Box 10 17 09, D-5600, Wuppertal-1, W. Germany
J Biochem (Tokyo); 90(6):1821-1824 1981

Hepatocytes from neo- and postnatal rats were successfully isolated, purified from non-hepatocytes (erythropoietic cells), and cultured in sufficient amounts to perform transport kinetics of nutrients. In the presence of sodium (Na+-dependent component) the amino acid uptake, measured with alpha-amino [1-14C]isobutyrate (AIB) as a tracer, was markedly enhanced during early postnatal phases. Kinetic analyses of these enhanced velocity data revealed the presence of a low-Km transport system in the range of amino acid concentration in which AIB mimics physiological properties. The system provides neonatal hepatocytes with a great potential for concentrating amino acids during the early postnatal stage, and may have considerable effect on membrane linked regulatory processes during liver development. (Author abstract) (19 Refs)

71. ANOREXIA IN CANCER: ANOTHER SPECULATION ON ITS PATHOGENESIS.

Theologides A
Univ. Minnesota Medical Sch., Minneapolis, MN
Nutr Cancer; 2(3):133-135 1981

The pathogenesis of anorexia as a result of cancer is reviewed. The presence of peptidergic pathways in the brain may act as neurotransmitters and have effect in the mechanism of regulation of food intake. It is proposed that cancers produce some peptides biologically active through which anorexia is induced. Following the injection of such peptides to experimental animals with cancer, the effect on food intake could be studied and the pathogenesis of anorexia could be understood better. (27 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Adachi T 37
Adamietz P 11
Aden DP 17
Albinsson A 61
Alfaro-Aceves L 20
Andersson G 61
Andersson LC 41
Anehus S 61
Antoniewica M 26
Aurell M 36
Bach FH 16
Bagley DH 68
Baici A 34
Banjar Z 3
Bannai S 65
Barel M 40
Barra R 64
Bellemann P 70
Benitez-Bribies 20
Bernshtein MI 58
Bethell DR 63
Bigner DD 27
Bigner SH 27
Bjorklund P 12
Bonner WM 1
Bramwell ME 44
Bredehorst R 11
Breslow JL 17
Briggs RC 3
Callahan GN 16
Capen CC 32
Cardillo A 67
Carney DN 15
Carraway R 23
Cassio D 47
Charriaut C 40
Chew DJ 32
Collin GR 64
Cooper RA 49
Corvol P 36
Cossu G 49
Cutroneo KR 55
Da Costa M 19
Daddona PE 45
Davis M 29
De Miguel C 24
De la Huerta-Sa 20
DeWys WD 59
Demant P 16
Deng JS 4
Dermer GB 22
Devaux C 36
DiPetrillo T 55
Dornish JM 53
Doss RC 39
Drozdz M 26
Duhl DM 3
Edde B 5
Eng LF 27
Erdos T 47
Fabrizi G 67
Fahrenkrug J 28
Falk J 14
Farallo A 67
Farbiszewski R 31
Feurle GE 23
Fisher C 19
Forssmann WG 23
Frade R 40
Frid IA 58
Friedkin M 9
Fries J 47
Fulton DS 62
Galen FX 36
Gallegos-Vargas 20
Gazdar AF 15
Gendler SJ 22
Genest J 36
Gingrich RD 44
Goberna R 24
Graf M 34
Gros F 5
Grube D 23
Guerrero JM 24
Gunther GR 51
Harden TK 39
Harris H 44
Heby O 61
Helmstaedter V 23
Henshaw EC 6
Hilz H 11
Hnilica LS 3
Holmes EW 10
Hood L 29
Hood L 35
Huikuri K 57
Hunt RC 42
Ishibashi M 48
Ishii T 65
Itabashi A 48
Javadpour N 68
Jeantet C 5
Johnson N 35
Jones TR 27
Jovine R 50
Jump DB 2
Kasuga K 21
Katayama S 48
Kenney DM 43
Kilczewska D 31
Kim S 29
Knowles BB 17
Kobata A 18
Kociba GJ 32
Kondo Y 48
Kosaka K 48
Kucharz E 26
Kuo AL 49
Landi FL 67
Lazo PA 60
Lea MA 64
Leduc M 33
Letarte M 14
Lindmo T 56
Lubich WP 62
Luning B 12
Maraldi NM 50
Marangos PJ 15
Marshall LM 42
Martin WJ 16
Marton LJ 62
Mastropaolo W 6
Mauritzon N 25
Mazzotti G 50
Menard J 36
Meuten DJ 32
Miani N 67
Midorikawa O 37
Mizumoto Y 48
Mort JS 33
Nabih I 66
Nathenson SG 16
Oaki A 21
Ohkawa K 21
Ohkawa R 21
Ohsawa N 48
Olofsson T 25
Olsson I 25
Ott MO 47
Page DL 3
Palka JA 13
Papa S 50
Parkman R 43
Parsons J 64
Patten P 29
Paul L 35
Pedersen JH 28
Pegg AE 52
Pegg AE 63
Perkins JP 39
Perrine S 43
Perry W 52
Pessano S 49
Pettersen EO 56
Pintado E 24
Pitha PM 54
Prestayko A 55
Preyre-Horta R 20
Prieto JC 24
Raj NB 54
Recklies AD 33
Remold-O'Donnel 43
Robinson SC 69
Rogers MJ 16
Roher HD 23
Ronning OW 56
Rosen FS 43
Rozengurt E 9
Rushton G 38
Rybkin MP 58
Rzeczycki W 13
Sala-Trepat JM 47
Salmanova EA 58
SanGiacomo TR 17
Sandquist D 7
Sandra A 7
Santi P 50
Schaffalitzky d 28

Schirrmacher V 16
Schold SC 27
Schor AM 38
Schor SL 38
Seglen PO 56
Serri F 67
Shoyab M 8
Sindelar WF 68
Sinn E 29
Skinner SL 36
Slankard J 35
Smith TC 69
Smith-Kielland 53
Soubrier F 36
Stadil F 28
Steinhagen-Thie 11
Sterling KM 55
Strauli P 34
Sugita Y 65
Tachibana Y 18
Takasaki Y 4
Takeuchi T 18
Tan EM 4
Theologides A 71
Tischbirek K 23
Todaro GJ 8
Tokes ZA 22
Tulikoura I 57
Vaheri A 30
Vartio T 30
Walker LE 16
Warren L 49
Warren TC 8
Weiss MC 47
Wielckens K 11
Wiklund B 12
Williams LR 7
Williams TH 7
Wilson CB 62
Winn B 38
Wouters M 44
Wu RS 1
Yamaji T 48
Yamashita K 18
Zahringer J 46
Zannis VI 17
Zdrodowska J 31

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In this issue:

- **NUCLEOPROTEINS 1-5**
- **CYTOPLASMIC AND CONTRACTILE PROTEINS 6-18**
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS 19-31**
- **BINDING/ RECEPTOR PROTEINS 32-34**
- **MEMBRANE/ SURFACE PROTEINS 35-41**
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS 42-46**
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM 47-51**
- **DIETARY AND NUTRITIONAL EFFECTS 52-53**
- **POLYAMINES - SYNTHESIS AND METABOLISM 54-56**
- **OTHER RELATED STUDIES 57-64**

NUCLEOPROTEINS

1. HISTONE H1 SUBFRACTIONS AND H1(0) TURNOVER AT DIFFERENT RATES IN NONDIVIDING CELLS.

Pehrson JR, Cole RD
Dept. Biochemistry, Univ. California, Berkeley, Berkeley, CA, 94720
Biochemistry; 21(3):456-460 1982

When cell division was inhibited in mouse neuroblastoma cells by high cell density or by serum deprivation, levels of histone H1(0) increased by a factor of three. Inhibition of cell division by butyrate increased H1(0) seven-fold. In addition to the increase in H1(0), changes were seen in the proportions of

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H1 subfractions whenever division was inhibited. These canges were only partly due to phosphorylation. When cell division was very active, a pulse of radioactive lysine labeled the various forms of H1 nearly in proportion to their protein mass. A chase of the radioactivity from such pulse-labeled cells showed a rapid turnover of the subfraction H1c, while the other forms of H1 turned over slowly, like the core histones. The inhibition of cell division reduced lysine incorporation into the subfractions of gel band H1a,b nearly as much as it reduced core histone synthesis, but H1c and H1(0) were not so drastically affected. Relative to incorporation into core histones, incorporation into H1c and H1(0) was 4-fold and 20-fold greater, respectively, in mitotically inactive cells. When this radioactivity was chased with unlabeled lysine, H1(0) was quite stable, but H1c and one of the H1 subfractions of the H1a,b band turned over rapidly. Unequal turnover of H1 subfractions and the difference in behavior between H1(0) and the more common H1's show how the recipe of H1 components can be varied even in the absence of DNA synthesis. These results are consistent with a chromatin repair process and also with a designed modulation of chromatin functions. (Author abstract) (32 Refs)

2. STUDIES ON PROTEIN KINASES IN TWO HUMAN RECTOCOLIC CELL LINES BY POLYACRYLAMIDE GEL ELECTROPHORESIS. EFFECT OF THE VASOACTIVE INTESTINAL PEPTIDE.

Mangeat P, Ait-Ahmed O, Marchis-Mouren G, Marvaldi J
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Biochim Biophys Acta; 714(2):320-330 1982

Electrophoresis and subsequent assay of the enzyme directly onto the gel has allowed a rapid and quantitative characterization of the cyclic AMP-dependent and -independent histone kinases, protamine, phosvitin and casein kinases in HT 29 and HRT 18 cells. The technique has been applied to soluble extracts from cytoplasmic and nuclear fraction prepared in the presence and absence of neutral detergent. A more precise identification of these enzymes has been possible by analyzing enzyme fractions obtained after ion-exchange chromatography of the above extracts. The protein kinase equipment of both cell lines was found to be identical (11 major components) but with different relative proportions of several enzymes. In cytoplasmic extracts: VIP activates only the Type I, cytosolic, (Band 4) and the Type II, membrane-bound, (Bands 6 and 8) cyclic AMP-dependent histone kinases. These enzymes account, respectively, for 34 and 55% of the total histone kinases in HT 29 and HRT 18 cells. The cyclic AMP-independent histone kinases (Band 1, 2, 5 and 7) also phosphorylate protamine; Band 5 was found to be much higher (4-fold) in HT 29 cells. In addition, two casein/phosvitin kinases have been identified in both cell lines with phosphorylating activity similar to the total histone kinases. In the nuclear extract two cyclic AMP-independent histone kinases have been found with electrophoretic mobility differing from the cytoplasmic enzymes. Also, two phosvitin/casein kinases specifically nuclear, due to their chromatographical and electrophoretical behavior, have been characterized. (Author abstract) (25 Refs)

3. HISTONE PROTEINS IN HELA S3 CELLS ARE SYNTHESIZED IN A CELL CYCLE STAGE SPECIFIC MANNER.

Marashi F, Baumbach L, Rickles R, Sierra F, Stein JL, Stein GS
Dept. Biochemistry, Univ. Florida Coll. Medicine, Gainesville, FL, 32610
Science; 215(4533):683-685 1982

The synthesis of histone proteins in G1 and S phase HeLa S3 cells was examined by two-dimensional electrophoretic fractionation of nuclear and total cellular proteins. Newly synthesized histones were detected only in S phase cells. Histone messenger RNA sequences, as detected by hybridization with cloned human histone genes, were present in the cytoplasm of S phase but not G1 cells. (Author abstract) (27 Refs)

4. ISOLATION OF NUCLEOSIDE PHOSPHOTRANSFERASES FROM CHROMATIN OF MORRIS HEPATOMA 9121 NUCLEI.

Lenger K
Instit fur Biochemische Endokrinologie, Medizinischen Hochschule Lubeck, Ratzeburger Allee 160, D-2400 Lubeck, W. Germany
Int J Biochem; 14(1):53-61 1982

Chromatin of Morris Hepatoma 9121 nuclei was obtained from isolated nuclei derived from tumors grown in male ACI rats. Thymidine phosphorylation activity of chromatin was identified in six peaks on diethylaminoethanol-cellulose chromatography. One nuclear enzyme (C) was separated into two subunits of approx 100,000 and 130,000 mol wt as estimated by calibration proteins. Density gradient centrifugation and Sephadex-G 150 gel filtration gave similar results, and confirmed the split nature of enzyme C. Peak activity of the enzyme was at pH 6.8-7.0, the best phosphate donors were AMP and ADP, and the preferred substrate was deoxyguanosine. Enzyme C is apparently a deoxyguanosine phosphotransferase and its reaction mechanism was suggested by the velocity of 14C-thymidine phosphorylation to be allosteric with positive cooperativity. (28 Refs)

5. THE GROWTH INHIBITION IN FRIEND ERYTHROLEUKEMIA CELLS INDUCES CYCLING OF PREEXISTING NONHISTONE PROTEINS.

Yancheva N, Djondjurov L
Institut po Molekularna Biologia, Bulgarska Akademiya na Naukite, BG-1113 Sofiya, Bulgaria
Eur J Biochem; 121(2):309-316 1982

The present investigation performed on Friend erythroleukemia cells provides evidence that the cellular reprogramming associated with transition of the cells from the growing to the resting state is accompanied by an intranuclear cycling of preexisting proteins migrating from the cytoplasm. The model system developed for this study affords an opportunity to follow these events in a conveniently short period. The flow microfluorometric analysis revealed that, with respect to DNA content, the quiescent nuclei exhibit distribution in G1, S and G2 phases, as do the nuclei of the control, exponentially growing cells. It was found further that the induction of quiescence arouses two waves of migration of preexisting cytoplasmic proteins toward the nucleus: an early one, during the transition to quiescence clearly showing density dependence, and a later one, in the established resting state. While the early-cycled proteins are undetectable in mass and once they have reached the nucleus have a short life time and, thus, could be considered as regulatory molecules, the late-cycled proteins accumulate within the nucleus and are associated most probably with structural reorganization of the resting nuclei; moreover, the late-cycled nonhistone proteins were found localized, at least partly, in the nuclear protein matrix structure. Control experiments confirmed that the early and late-cycled proteins have been synthesized in the cytoplasm in the proliferative state but are cycled intranuclearly only if the resting state has been induced. When finally the resting cells were stimulated to proliferate, the accumulated presynthesized and newly synthesized nonhistone proteins undergo a rapid degradation which suggests that the new cellular program may require a complete elimination of the 'resting' nonhistone proteins. (Author abstract) (45 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

6. CAMP RECEPTOR PROTEINS AND PROTEIN KINASES IN HUMAN LYMPHOCYTES: FUNDAMENTAL ALTERATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS.

Weber W, Schwoch G, Wielckens K, Gartemann A, Hilz H
Physiologisch-Chemisches Institut, der Universitat Hamburg, Universitats-Krankenhaus Eppendorf, Martinistrabe 52, D-2000 Hamburg 20, W. Germany
Eur J Biochem; 120(3):585-592 1981

To study the significance of cAMP receptor proteins and protein kinase activities in tumor dys-differentiation, peripheral blood lymphocytes from normal donors and from patients with chronic lymphocytic leukemia were isolated by Percoll gradient centrifugation, and analyzed. Both normal and tumor lymphocyte populations were devoid of cell proliferation. Normal human lymphocytes contained about equal activities of histone kinase and 'casein kinase'. The tumor lymphocytes, however, exhibited drastically reduced values of all three protein kinase activities, the most pronounced decrease being observed with the 'casein kinase'. Total high-affinity cAMP-binding sites (= regulatory subunits R of cAMP-dependent protein kinases Type I and II) in leukemic lymphocytes were also strongly reduced. The findings in lymphocytes of chronic lymphocytic leukemia cannot be explained by the fact that they are B-type lymphocytes. Control experiments revealed that normal B cells do not differ significantly from total blood lymphocytes with respect to the parameters mentioned above. Biochemical correlates of the dys-differentiated status of these non-proliferating cells from patients with chronic lymphocytic leukemia, then, are represented by reduced levels of protein kinases, of basal cAMP as well as by changes in the R-protein pattern. The data indicate that both protein kinases Type I and Type II may relate to the state of differentiation, independently from cell proliferation. (57 Refs)

7. PHOSPHORYLATION OF SYNTHETIC PEPTIDES BY A TYROSINE PROTEIN KINASE FROM THE PARTICULATE FRACTION OF A LYMPHOMA CELL LINE.

Casnellie JE, Harrison ML, Pike LJ, Hellstrom KE, Krebs EG
Howard Hughes Medical Inst. Lab., Dept. Pharmacology, Univ. Washington, Seattle, WA, 98195
Proc Natl Acad Sci USA; 79(2):282-286 1982

The particulate fraction from a lymphoma cell line, LSTRA, was found to contain an apparent high level of tyrosine protein kinase activity. When this fraction was incubated with [gamma-32P]ATP in the presence of 10 mM MnCl2, hydrolyzed, and assayed, 70-80% of the radioactivity recovered in phosphoamino acids was in phosphotyrosine. Gel electrophoresis of the proteins showed that a large portion of the 32P was in a single protein with a mol wt of approx 58,000. The phosphorylated residue in this protein was identified as phosphotyrosine. Detergent extracts of the particulate fraction from LSTRA cells contained both the Mr 58,000 protein and the enzyme responsible for its phosphorylation. These extracts were found to catalyze the phosphorylation of the tyrosine residue in the synthetic peptide, Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly, corresponding to the sequence around the tyrosine that is phosphorylated in pp60src; the Km for the peptide in this reaction was 5 mM. High-performance liquid chromatography was used to assay for this phosphorylation. A second peptide was synthesized that contained two additional arginine residues whose presence permitted the phosphorylation of the peptide to be measured by a simple assay using phosphocellulose paper. The Km for this peptide was 3-4 mM, indicating that the presence of the additional arginine residues did not alter the apparent affinity of the kinase for the peptide. (Author abstract) (24 Refs)

8. STIMULATION OF TYROSINE-SPECIFIC PHOSPHORYLATION BY PLATELET-DERIVED GROWTH FACTOR.

Ek B, Westermark B, Wasteson A, Heldin CH
Biomedical Center, Inst. of Medical and Physiological Chemistry, Uppsala Univ., Uppsala, Sweden
Nature; 295(5848):419-420 1982

The ability of platelet-derived growth factor (PDGF) binding to cellular receptors to stimulate kinase activity was studied in membranes prepared from cultured human foreskin fibroblasts or glial cells. PDGF stimulated the incorporation of 32P into membrane proteins of both fibroblasts a nd glial cells, promoting, in particular, the phosphorylations of a 175,000-molecular wt (Mr) and a 130,000-Mr protein, in comparison with the control incubation, in which no growth factor was added. Slight effects of PDGF were also seen in other components of the autoradiogram, associated with both increased and decreased phosphorylation. In the presence of epidermal growth factor (EGF), 32P incorporation increased preferentially in a 170,000-Mr protein of the fibroblast membrane preparation and in both a 170,000-and a 150,000-Mr component of the glial cell membrane preparation. The results indicated that different proteins serve as substrates for the kinases stimulated by the two factors. In membranes of the A431 epithelial cell line, only EGF appreciably increased phosphorylation. The specificity of the PDGF-stimulated kinase activity was studied in relation to the type of phospho-accepting amino residue involved. The mechanisms of action of EGF and PDGF are discussed. (34 Refs)

9. ORNITHINE OXOACID AMINOTRANSFERASE FOUND IN AH 130 ASCITES HEPATOMA CELLS.

Matsuzawa T, Sugimoto N, Sobue M, Ishiguro I
Dept. Biochemistry, Fujita-Gakuen Univ. Sch. Medicine, Toyoake, Aichi 470-11, Japan
Biochim Biophys Acta; 714(2):356-360 1982

We found heterogenous ornithine oxoacid aminotransferase (L-ornithine: 2-oxo-acid aminotransferase, EC 2.6.1.13) in rat ascites hepatoma AH 130 cells. Compared with enzymes from normal rat tissues, this heterogenous enzyme showed larger Km values for 2-oxoglutarate, a different elution-profile upon affinity chromatography with 2-oxoglutarate, more anionic mobility upon polyacrylamide gel electrophoresis, and a clearly different salting-out property upon ammonium sulfate fractionation. Similar heterogeneity of this aminotransferase was found in human cancer cells. (Author abstract) (25 Refs)

10. OCCURRENCE IN VARIOUS MAMMALIAN CELLS AND TISSUES OF CA(2)+-ACTIVATED PROTEASE SPECIFIC FOR THE INTERMEDIATE-SIZED FILAMENT PROTEINS VIMENTIN AND DESMIN.

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Eur J Cell Biol; 26(1):61-67 1981

Several mammalian cell lines propagated in suspension and monolayer culture and some normal and cancerous tissues from rat, hamster and cat were screened for the presence of the Ca++-activated protease specific for the intermediate-sized filament protein vimentin. Gel permeation chromatography on Sephacryl S-300 of postnuclear supernatants, and sucrose density gradient centrifugation of extracts from Triton X-100-resistant residual cell structures revealed the presence of the enzyme in all cells and tissues tested. Its apparent mol wt amounted to 100,000. Except in the cases of a spontaneous rat lung tumor and a rat hepatocellular carcinoma induced by diethylnitrosamine, most of the enzyme was released into the postnuclear supernatant during cell or tissue extraction, indicating that it is of cytoplasmic origin. There was no correlation between the enzyme level and the vimentin content of cells and tissues. Rat and hamster liver as well as cat kidney, in which vimentin has not been detected by polyacrylamide gel electrophoresis, were relatively rich in the Ca++-activated protease. The experimental results point at the widespread, if not general, occurrence of the enzyme in mammalian cells. (Author abstract) (33 Refs)

11. HUMAN LEUKEMIA AND NORMAL LEUKOCYTES CONTAIN A SPECIES OF IMMUNOREACTIVE BUT NONFUNCTIONAL DIHYDROFOLATE REDUCTASE.

Rothenberg SP, Iqbal MP
Dept. Medicine, Section Hematology/Oncology, Brooklyn Veterans Admin., Brooklyn, NY, 11209
Proc Natl Acad Sci USA; 79(2):645-649 1982

A quantitative radioimmunoassay has been developed for human dihydrofolate reductase (tetrahydrofolate dehydrogenase; 5,6,7,8-tetrahydrofolate:NADP+ oxidoreductase EC 1.5.1.3), by using antiserum raised in rabbits against the active enzyme purified from calf liver. An immunoreactive protein could be identified in the cytoplasm of chronic myelogenous leukemia cells, which contained no functional dihydrofolate reductase activity. Its concentration was stoichiometric to the vol of cytoplasm assayed and paralleled the standard curve obtained with purified enzyme, indicating that this protein in the human cells is antigenically similar to the homologous antigen. The concentration of this immunoreactive protein in the cytoplasm of human leukemia and normal leukocytes in all instances greatly exceeded the concentration of functional dihydrofolate reductase, which was measured by the binding of [3H]methotrexate. This nonfunctional immunoreactive protein in the cytoplasm and cytosol from two different samples of chronic myelogenous leukemia cells analyzed by gel filtration had an apparent mol wt of 41,000, which is twice the mol wt of the functional enzyme. (Author abstract) (33 Refs)

12. PURIFICATION AND PROPERTIES OF A RAT LIVER PROTEIN THAT SPECIFICALLY INHIBITS THE PROLIFERATION OF NONMALIGNANT EPITHELIAL CELLS FROM RAT LIVER.

McMahon JB, Farrelly JG, Iype PT
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Proc Natl Acad Sci USA; 79(2):456-460 1982

An inhibitor of cell proliferation was purified from rat liver by alcohol precipitation, ultrafiltration, and DEAE-cellulose chromatography. The hepatic proliferation inhibitor was shown to be pure by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, analytical isoelectric focusing, and high-performance liquid chromatography. The hepatic proliferation inhibitor was found to have a molecular weight of 26,000 and an isoelectric point of 4.65. This protein inhibited the proliferation of nonmalignant rat liver cells in culture, and removal of the protein reversed the inhibition produced by low doses. It exerted no effect on the proliferation of malignant rat liver cells. (Author abstract) (31 Refs)

13. EUKARYOTIC MRNA CAPPING ENZYME-GUANYLATE COVALENT INTERMEDIATE.

Venkatesan S, Moss B
Lab. Biology of Viruses, Natl. Inst. Allergy and Infectious Diseases, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(2):340-344 1982

Incubation of HeLa cell mRNA guanylyltransferase (GTP:mRNA guanylyltransferase, EC 2.7.7.50) with [alpha-32P]GTP and a divalent cation in the absence of an RNA acceptor results in the formation of a covalent enzyme-guanylate complex. The complex, after purification by phosphocellulose chromatography, can transfer its bound GMP moiety to pyrophosphate, regenerating GTP, or to the 5'-diphosphate end of poly(A), forming a cap structure G(5')pppA(pA)n. The GMP-polypeptide has a mol wt of 65,000 and is stable to heating in the presence of sodium dodecyl sulfate. On the basis of the alkali-stable and acid-labile nature of the bond and its susceptibility to nucleophilic attack by hydroxylamine at low pH, the GMP-polypeptide linkage appears to be a phosphoamide bond. After digestion with trypsin, a single GMP-peptide was resolved by two-dimensional electrophoresis and chromatography. (Author abstract) (29 Refs)

14. STABILITY OF HEXOKINASE II IN VITRO AND IN ASCITES TUMOR CELLS.

Rose IA, Warms VB
Inst. for Cancer Res., Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA, 19111
Arch Biochem Biophys; 213(2):625-634 1982

Contribution of stability properties of isolated hexokinase II (HK) to HK stability in the intact cell was studied. Ehrlich-Lettre hyperdiploid ascites (ELD) cells were maintained by weekly transfer in ICR white mice. HK of ELD is almost exclusively the thermolabile HK form. HK is temperature sensitive (t1/2 approx 5 min at 30 C) but is stabilized in whole ascites tumor cells lacking glucose. Binding of HK to the mitochondrial membrane causes stabilization. K+ which has no effect on any of the catalytic properties of HK increases the half-time of thermal inactivation 15-fold. NADPH-thioredoxin reductase system causes stabilization and reactivation of HK. The disulfide form of HK is not stabilized by glucose and K+ and decays irreversibly with loss of the major antigenic determinants of the native HK. Glucose or K+ prevents but does not reverse the loss of HK activity. Lactic dehydrogenase activity was not diminished. The reversibly oxidized enzyme is a good target for the cell's protein breakdown system. Sodium arsenite, a reagent that makes linkages to neighboring sulfhydryl groups of proteins, was able to inactivate HK in vitro and in the cell. The rate of change to unreactivatable form was the same in both cases and similar to the rate of decay of the metastable, oxidized form in vitro. The greater stability of the arsenite form in the cell may reflect an additional effect of arsenite in inhibiting the cell's protein breakdown system. (30 Refs)

15. PROTEIN PATTERNS AS CODES FOR TUMOR IDENTIFICATION.

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Anal Biochem; 118(2):392-395 1981

Particulate-fraction and soluble-fraction proteins from examples of breast carcinoma, Hodgkin's lymphoma, and colon adenocarcinoma and their corresponding normal tissues were resolved on sodium dodecyl sulfate-polyacrylamide gels. The Coomassie blue-stained protein patterns were quantitated using a scanning densitometer and were reduced to simple three-part numerical codes. The codes were (a) specific for each tissue and (b) in the examples of lymphoma and colon adenocarcinoma, indicated a strong similarity between tumors and their respective normal tissues. (Author abstract) (15 Refs)

16. MICROTUBULE-ASSOCIATED PROTEINS OF HELA CELLS: HEAT STABILITY OF THE 200,000 MOL WT HELA MAPS AND DETECTION OF THE PRESENCE OF MAP-2 IN HELA CELL EXTRACTS AND CYCLED MICROTUBULES.

Weatherbee JA, Sherline P, Mascardo RN, Izant JG, Luftig RB, Weihing RR

Worcester Foundation Experimental Biology, Shrewsbury, MA, 01545

J Cell Biol; 92(1):155-163 1982

One of the major groups of microtubule-associated proteins (MAPs) found associated with the microtubules isolated from HeLa cells has a mol wt of just over 200,000. Previous work has demonstrated that these HeLa MAPs are similar in several properties to MAP-2, one of the major MAPs of mammalian neural microtubules, although the two types of proteins are immunologically distinct. The 200,000 mol wt HeLa MAPs have now been found to remain soluble after incubation in a boiling water bath and to retain the ability to promote tubulin polymerization after this treatment, two unusual properties also shown by neural MAP-2. This property of heat stability has allowed the development of a simplified procedure for purification of the 200,000 HeLa MAPs and has provided a means for detection of these proteins, even in crude cell extracts. These studies have also led to the detection of a protein in crude extracts of HeLa cells and in cycled HeLa microtubules which has been identified as MAP-2 on the basis of (a) comigration with calf brain MAP-2 on SDS PAGE, (b) presence in purified microtubules, (c) heat stability, and (d) reaction with two types of antibodies prepared against neural high mol wt-MAPs, one of these a monoclonal antibody against hog brain MAP-2. MAP-2, although present in HeLa cells, is at all stages of microtubule purification a relatively minor component in comparison to the 200,000 HeLa MAP's. (Author abstract) (48 Refs)

17. A MICROTUBULE-ASSOCIATED PROTEIN IN THE MITOTIC SPINDLE AND THE INTERPHASE NUCLEUS.

Izant JG, Weatherbee JA, McIntosh JR

Dept. Molecular, Cellular and Developmental Biology, Univ. Colorado at Boulder, Boulder, CO, 80309

Nature; 295(5846):248-250 1982

Spindle regulatory factors were studied using monoclonal antibodies prepared against a microtubule-associated protein fraction from HeLa cells. Lymphocyte hybridoma methodology was used to identify specific mitotic spindle components. Results support the hypothesis that a subset of mitotic spindle components is sequestered in the nucleus during interphase and that nuclear envelope breakdown initiates or augments spindle assembly by releasing important factors into the cytoplasm. (18 Refs)

18. CHARACTERIZATION OF STRUCTURAL PROPERTIES FOR MORPHOLOGICAL DIFFERENTIATION OF MELANOSOMES: II. ELECTRON MICROSCOPIC AND SDS-PAGE COMPARISON OF MELANOSOMAL MATRIX PROTEINS IN B16 AND HARDING PASSEY MELANOMAS.

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J Invest Dermatol; 78(1):76-81 1982

This study compared the qualitative and quantitative differences of the melanosomal matrix proteins between the ellipsoidal-lamellar melanosomes of B16 melanoma and the spherical-granular melanosomes of Harding Passey (HP) melanoma by electron microscopy and SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis). The purity of the melanosomes at each step of the sample preparations was assayed by per cent distribution of cytoplasmic marker enzymes and proteins. Characterization of the melanosomal matrix was carried out in material purified by the two steps of sucrose density gradient ultracentrifugation and membrane dissociation with BRIJ-35. Eighty-three percent of the matrix proteins were solubilized from both B16 and HP by guanidine HCl (GH), which also caused marked ultrastructural changes in different ways. The inner matrix of HP completely disintegrated into fine grains whereas the matrix of B16 showed a great loss in electron density and was degraded, though its basic framework remained unchanged. Under SDS-PAGE, the melanosomal matrix was dissociated into 14 polypeptide bands with respect to size and charge density, 10 of which (63-85% of total proteins solubilized by GH) were common to both B16 and HP. A significant quantitative difference was noted in the relative amounts of these common proteins. The remaining four were unique in mol wt to each form of melanosome though their relative amounts were low. It is suggested that these chemical differences, ie, quantitative and qualitative, of the matrix proteins are responsible for the ultrastructural differentiation of melanosomes. (Author abstract) (21 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

19. MEMBRANE-BOUND AND SECRETED IGA CONTAIN STRUCTURALLY DIFFERENT ALPHA-CHAINS.

Sitia R, Kikutani H, Rubartelli A, Bushkin Y, Stavnezer J, Hammerling U
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J Immunol; 128(2):712-716 1982

Three different forms of alpha-chains are synthesized by BFO.3 and 615.2, two cloned cell lines derived from the murine B lymphoma I.29. The three forms of alpha-chains differ in size, pI, cellular location, and rate of turnover. They were identified by means of lactoperoxidase-catalyzed radioiodination, internal 14C or 35S labeling, and immunofluorescence techniques as membrane-bound(alpham), secreted (alphas), and intracellular (alphaic) proteins. Comparison of immunoglobulin products of the two lymphoma lines with those of a hybridoma cell line, Id 150, which secretes IgA of the I.29 idiotype but lacks membrane IgA, confirmed the assignments of alpham, alphas, and alphaic. Results of biosynthetic labeling of BFO/3, 615.2, and Id 150 in the presence and absence of tunicamycin suggest that the difference in mol wt and charge observed between alpham and alphas can be attributed to differences in primary amino acid structure rather than different degrees of glycosylation. (Author abstract) (28 Refs)

20. DEFECTIVE BASAL LAMINA FORMATION BY TRANSFORMED MAMMARY EPITHELIAL CELLS: A REDUCED EFFECT OF COLLAGEN ON BASAL LAMINA (HEPARIN SULFATE-RICH) PROTEOGLYCAN DEGRADATION.

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J Cell Physiol; 110(1):56-62 1982

Low passage (LP, continuous basal lamina formed) and high passage (HP, scant basal lamina formed) strains of the NMuMG mouse mammary epithelial cell line were compared in terms of the nature of the 35SO4-proteoglycans accumulating and the 35S-glycosaminoglycan (GAG) turnover in cultures on collagen and plastic substrata. After 48 hr labeling on collagen, LP and HP cells had accumulated similar amounts of 35S-GAG, but HP cells had deposited less 35S-GAG on the substratum. Chasing for 48 hr revealed that the 35S-GAG produced by both lines are nearly equally stable. Sepharose CL-4B chromatography showed that the substratum-associated proteoglycan fractions, especially the basal lamina proteoglycan fraction, are deposited by the HP cells. When cultured on plastic the LP and HP monolayers accumulated similar amounts of 35S-GAG after 48 hr labeling and rapidly lost similar proportions after 24-48 hr chase. The major chromatographic proteoglycan fractions were lost from the plastic at similar rates, indicating that GAG metabolism of the two cell types does not differ on plastic. The 35S-proteoglycans accumulating in HP cultures contain more heparin sulfate and less chondroitin sulfate than those of LP cultures. Label-chase kinetic experiments showed that the reduced ability of HP cells to accumulate substratum-associated proteoglycans on collagen is due to their rapid degrading of a larger proportion of newly synthesized GAG. Experiments on plastic substrates showed that degradation rates of newly synthesized 35S-GAG are similar, and initially are more rapid than on collagen. The mechanism accounting for the inability of HP cells to respond to collagen by slowing proteoglycan degradation may involve the greater amount of heparin sulfate in HP cells or some unknown factor. (26 Refs)

21. THE MORPHOLOGY OF BASEMENT MEMBRANE FORMATION.

Merker HJ, Barrach HJ
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Eur J Cell Biol; 26(1):111-120 1981

A transplantable, basement-membrane (BM)-producing murine tumor was investigated using immunofluorescence and electron microscopy. Collagen Type IV, fibronectin, laminin, and a BM-proteoglycan (BM-1) could be discriminated in the intercellular substance (ICS). The occurrence of these substances and the electron microscopic findings speak for a comparability of the ICS of the tumor with the BM or normal tissues. Hence, a model has become available to study the morphology of BM-formation. The BM-producing cells are characterized by numerous, often enlarged cavities of the rough endoplasmic reticulum (RER) and a large Golgi complex. The BM-material is released into the ICS via prosecretion and electron-dense secretion granules. The morphology of the extracellular BM-material depends upon the fixation technique. After addition of tannic acid, typical BM-lamellae are formed, which in most cases show a granular structure. This is due to shrinkage and attachment of glycoproteins to the collagen skeleton. After ruthenium red or after glutaraldehyde fixation, the extracellular matrix consists of homogeneously distributed filamentous material with only few granular structures. This situation corresponds to the small amount of acid proteoglycans in the BM. The present findings facilitate the evaluation of BM-formation in other cell types. (Author abstract) (51 Refs)

22. PURIFICATION OF A LYMPHOID CELL LINE PRODUCT WITH LEUKOCYTE INHIBITORY FACTOR ACTIVITY.

Meshulam DH, Blair HE, Wong BL, Charm S, Minowada J, Rocklin RE
Enzyme Center, Tufts Univ. Sch. Medicine, Boston, MA, 02111
Proc Natl Acad Sci USA; 79(2):601-605 1982

Leukocyte inhibitory factor (LIF), a lymphokine that inhibits the random and directed migration of polymorphonuclear (PMN) leukocytes, was purified from a human non-T, non-B leukemia cell line (Reh). From 10 liters of serum-free supernatant, 1.3 ug of protein with LIF activity was obtained by the sequential use of affinity chromatography with concanavalin A-Sepharose, hydrophobic chromatography with hexylagarose, and gel filtration chromatography. The specific activity of LIF recovered represented an 80,000-fold purification over that of the initial crude serum-free supernatants, and the preparation at that point was estimated to be 80-90% pure. To both assess the purity of the preparation and provide a further purification step, Reh LIF activity recovered by the above procedures was subjected to isoelectric focusing. One major stainable protein band was identified; its isoelectric point was pH 5.4-5.5. Gels run in parallel for recovery of biologic activity revealed only one region (pH 5.4-5.5) with ability to inhibit PMN leukocyte migration. Iodination of Reh LIF resulted in a loss of biologic activity, but isoelectric focusing of this material revealed one major 125I-labeled band (pH 5.1) and several minor bands. The coincidence of biologic LIF activity with one stainable protein band as identified by isoelectric focusing implies that the final product may be homogeneous. (Author abstract) (21 Refs)

23. HUMAN MONOCLONAL IGM WITH AUTOANTIBODY ACTIVITY AGAINST INTERMEDIATE FILAMENTS.

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Proc Natl Acad Sci USA; 79(2):446-450 1982

Monoclonal IgMs from two patients with Waldenstrom macroglobulinemia were found to react with intermediate filaments. This was shown by (a) immunostaining of various tissues and cultured cells and (b) immunological characterization of the reactive antigen after blotting of polypeptides separated from total cell extracts by gel electrophoresis or purified intermediate filaments on nitrocellulose sheets. One monoclonal IgM had an activity directed only against vimentin, whereas the other reacted with four different classes of intermediate filaments-vimentin, desmin, glial fibrillary protein, and keratins. All of the reactivity of the latter IgM was absorbed by purified vimentin, suggesting that different classes of proteins of intermediate filaments share common antigenic determinant(s). The significance of such autoantibody activity of human monoclonal IgM is discussed in the light of the startling frequency of IgM anti-intermediate filaments antibodies in various diseases. (Author abstract) (32 Refs)

24. ECTOPIC PRODUCTION OF PLACENTA-'SPECIFIC' TISSUE PROTEINS (PP5 AND PP11) BY MALIGNANT BREAST TUMORS.

Inaba N, Renk T, Daume E, Bohn H
(c/o Bohn) Res. Laboratories, Behringwerke AG, Postfach 1140, D-3550 Marburg (Lahn), W. Germany
Arch Gynecol; 231(1):87-90 1981

An enzyme-bridge immunoperoxidase technique was used to demonstrate the production of two placenta-'specific' tissue proteins, PP5 and PP11 in sections of the breast cancers of 16 patients. The specific antisera against human PP5 and PP11 were raised by immunizing rabbits with the purified antigens. An IgG fraction of goat antiserum against rabbit immunoglobulin was used as the second antibody. Peroxidase-rabbit antiperoxidase (PAP) complex was then applied. A slightly modified Mason PAP method was used to demonstrate the tissue antigens. Clear positive staining for PP5 was observed in 10/16 (62.5%) breast cancers, and for PP11 in 7/15 (46.7%). Positive staining was only observed in the cytoplasm. All of the specimens positive for PP11 were also positive for PP5; all six specimens negative for PP5 were also negative for PP11. Positive staining was more frequent in cells found in the periphery of the cancer mass than those in the center. No staining was observed in apparently normal breast tissues and histiocytes in the tissues and/or in the vessels. These determinations in sera might be of use in screening for breast cancer and for monitoring therapy or detecting metastases in patients with established disease. (17 Refs)

25. THE SEARCH FOR PROGESTERONE-DEPENDENT PROTEINS SECRETED BY HUMAN ENDOMETRIUM.

MacLaughlin DT, Sylvan PE, Richardson GS
Vincent Memorial Hosp., Boston, MA
Adv Exp Med Biol; 138:113-131 1982

Studying the mechanism of action of progesterone on human endometrium, analysis of uterine luminal fluid was carried out to identify gene products for this steroid. Results showed that human uterine fluid contains a group of proteins not present in serum that are detectable mainly in the secretory phase of the menstrual cycle and that the proteins may be progesterone-induced products of human endometrial secretion. (75 Refs)

26. PROTEINS OF HUMAN URINE. II. IDENTIFICATION BY TWO-DIMENSIONAL ELECTROPHORESIS OF A NEW CANDIDATE MARKER FOR PROSTATIC CANCER.

Edwards JJ, Anderson NG, Tollaksen SL, Von Eschenbach AC, Guevara J

Molecular Anatomy Program, Div. Biological and Medical Res., Argonne Natl. Lab., Argonne, Il, 60439

Clin Chem; 28(1):160-163 1982

A protein series common to the urine and prostatic tissue of 16 of 17 patients with prostatic adenocarcinoma has been identified by high-resolution two-dimensional gel electrophoresis. These proteins, designated PCA-1, have a relative molecular mass in sodium dodecyl sulfate of about 40,000. Analyses of urines from eight age-matched controls, seven patients with other types of urogenital malignancies, two patients with benign prostatic hyperplasia, and five patients with malignancies not associated with the urogenital system failed to show PCA-1 in the patterns. These preliminary findings suggest that this protein should be systematically investigated as a candidate marker for prostatic adenocarcinoma in man. (Author abstract) (23 Refs)

27. BASAL LAMINA GLYCOPROTEINS LAMININ AND TYPE IV COLLAGEN ARE ASSEMBLED INTO A FIN E-FIBERED MATRIX IN CULTURES OF A TERATOCARCINOMA-DERIVED ENDODERMAL CELL LINE.

Leivo I, Alitalo K, Risteli L, Vaheri A, Timpl R, Wartiovaara J

Dept. Electron Microscopy, Pathology, Univ. Helsinki, Helsinki, Finland

Exp Cell Res; 137(1):15-23 1982

Extracellular matrix glycoproteins synthesized and deposited by a mouse teratocarcinoma-derived endodermal cell line (PYS-2) in culture were analyzed by metabolic labelling and immunochemical methods, and the matrix structure was studied by immunofluorescence and electron microscopy. PYS-2 cells secreted two major high-mol wt glycoproteins, laminin and Type IV collagen, which were deposited in apparently unprocessed form under the cells into a lamellar matrix composed of a loose network of fine fibrils and attached dense grains. The cells did not synthesize detectable amounts of fibronectin, but the matrix was found to bind fibronectin from the culture medium. The matrix structure was sensitive to bacterial collagenase indicating a role for Type IV collagen in matrix integrity. The PYS-2 matrix which contains defined basal lamina glycoproteins provides possibilities for in vitro studies on the organization of deposited basal lamina components. (Author abstract) (47 Refs)

28. RESPONSES OF NORMAL AND MALIGNANT CELLS TO COLLAGEN, COLLAGEN-DERIVED PEPTIDES AND THE C5-RELATED TUMOR CELL CHEMOTACTIC PEPTIDE.

Wass JA, Varani J, Piontek GE, Ward PA, Orr FW
Dept. Pathology, Univ. Michigan Medical Sch., Ann Arbor, MI, 48109
Cell Differ; 10(6):329-332 1981

The chemotactic response of normal fibroblasts and metastasizing and nonmetastasizing murine fibrosarcoma cells to collagen and collagen degradation products was examined and compared with the response to the fifth complement component (C5) chemotactic peptide. Normal fibroblasts and nonmetastasizing fibrosarcoma cells responded to lathyritic chick collagen, glycyl-L-propyl-L-hydroxyproline, and glycyl-L-hydroxyproline, but not to the C5 peptide. In contrast, metastasizing fibrosarcoma cells responded to the C5 peptide but not to collagen or collagen-related peptides. None of the cells responded to L-prolylhydroxy-L-proline. Results emphasize similarities between nonmetastasizing and normal cells and indicate a functional difference between these cells and metastasizing cells. (16 Refs)

29. FUNCTIONAL ALPHA1 PROTEASE INHIBITOR PRODUCED BY A HUMAN HEPATOMA CELL LINE.

Glasgow JE, Bagdasarian A, Colman RW
Albert Einstein Medical Center, Korman Building, York and Tabor Roads, Philadelphia, PA, 19141
J Lab Clin Med; 99(1):108-117 1982

Alpha1 protease inhibitor antigen was identified in the culture medium of the human ascites hepatoma cell line SK-HEP-1. Trypsin inhibitory activity and alpha1pI antigen accumulated in serum-free medium concomitantly over a period of several days. Radioactive alpha1PI antigen was de tected in conditioned medium from cultures supplemented with 35S-L-methionine, indicating synthesis and release of the protein. alpha1PI antigen in conditioned medium appeared to be antigenically identical to that in human plasma, and the newly synthesized (radiolabeled) antigen co-migrated with plasma alpha1PI after immunoelectrophoresis or SDS-polyacrylamide gel electrophoresis. Moreover, evidence is presented that the synthesized inhibitor exhibits functional activity, since the 35S-labeled alpha1PI in conditioned medium complexes with trypsin. We concluded that SK-HEP-1 cells in culture produce functionally active alpha1PI which may be identical to that in plasma. (Author abstract) (25 Refs)

30. BASEMENT MEMBRANE RESEARCH.

Risteli L, Risteli J
Dept. Medical Biochemistry, Univ. Oulu, Oulu, Finland
Med Biol; 59(4):185-189 1981

Recent progress in basement membrane research is summarized. The interaction of metastatic tumor cells with basement membranes derives importance from the protective barrier against invasion provided by the membrane. Tumor cells have been shown to secrete a protease which degrades type IV collagen; degree of secretion correlates with malignant potential of the cell. (no Refs)

31. ESTRAMUSTINE BINDING IN RAT, BABOON AND HUMAN PROSTATE MEASURED BY HIGH PRESSURE LIQUID CHROMATOGRAPHY.

Kirdani RY, Corrales JJ, Hoisaeter PA, Karr JP, Murphy GP, Sandberg AA
Roswell Park Memorial Inst., Buffalo, NY, 14263
Steroids; 37(5):471-484 1981

High pressure liquid chromatography (HPLC) was used to determine 3H-estramustine (estradiol-17beta3N-bis-[2-chlorethyl] carbamate), 3H-17beta-hydroxy-5-alpha-androstan-3-one (3H-dihydrotestosterone or 3H-DRT), 3H-estradiol-17beta (3H-E2) and 3H-3beta-hydroxy-5-pregnen-20-one [3H-pregnenolone] binding in 50 ul of cytosol utilizing a column which separates proteins in the mol wt range of 2,000 to 70,000 daltons. The rat prostate contains a protein in considerable concentration and with the highest affinity for estramustine (375,000 dpm 3H-estramustine per mg cytosol protein) among the substances tested. Operationally, we have named this protein 'estramustine binding protein' (EBP), though it is very likely similar to other previously described prostatic proteins eg, alpha-protein, prostatein prostatic binding protein). The sensitivity of the HPLC method disclosed EBP-like proteins, but in much lesser concentratons, in some of the other tissues tested. The concentration of these proteins in the human and baboon prostates was much lower (av for the baboon cranial lobe 4,800 dpm/mg cytosol protein, with a somewhat higher value for the caudal lobe) than that in the rat gland. The amount of EBP-like protein was higher in prostatic cancer than in that of benign prostatic hypertrophy (range 9,350-25,900 vs 2,200-18,900 dpm/mg cytosol protein). In the human, the highest value was found in one normal prostate tested (106,000 dpm/mg cytosol protein). (Author abstract) (13 Refs)

BINDING/ RECEPTOR PROTEINS

32. RETINOIC ACID-BINDING PROTEIN IN HUMAN SQUAMOUS CELL CARCINOMAS OF THE ORL REGION.

Bichler E, Daxenbichler G
Univ. HNO-Klinik Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria
Cancer; 49(4):619-622 1982

In some squamous cell carcinoma of the otorhinolaryngologic region with different grades of differentiation, a protein was found that specifically binds vitamin A acid. In 28 of 37 tumors, the retinoic acid-binding sites were found in significant amounts, according to the authors' data. Areas with metastases showed a lower incidence of retinoic acid-binding, whereas in all normal epiglottis and vocal cord tissue specimens the binding was present. The possible significance of the protein-binding for the biologic effect of the vitamin A acid is discussed. (Author abstract) (28 Refs)

33. HIGH AFFINITY BINDING SITES ON PLASMA MEMBRANE OBTAINED FROM THE LYMPHOBLASTOID CULTURED 1301 CELL LINE FOR HIGHLY RADIOACTIVE SERUM THYMIC FACTOR.

Gastinel LN, Pleau JM, Dardenne M, Sasaki A, Bricas E, Morgat JL, Bach JF

INSERM U 25, Hopital Necker, 161 rue de Sevres, 75015 Paris, France

Biochim Biophys Acta; 684(1):117-126 1982

The interaction of the synthetic serum thymic factor (FTS, facteur thymique serique) with a plasma membrane preparation of human T lymphocytes from the lymphoblastoid T cell line 1301 was studied using 3H-labelled FTS (specific activity 120 Ci/mmol). The binding is temperature dependent and function of the concentration of both 3H-labelled FTS and membrane proteins. At 37 C, using 1 nM of 3H-labelled FTS a steady state is observed within 80 min. The binding is reversible, specific and saturable. Scatchard analysis reveals the existence of at least two binding sites with respective Kd of the order of 0.516 +-0.2 nM and 110 +-27.8 nM with concentrations of 0.186 +-0.045 pmol and 2.026 +-0.367 pmol per mg of membrane protein. (Author abstract) (36 Refs)

34. CYCLIC AMP-BINDING PROTEIN AND ESTROGEN RECEPTOR: ANTAGONISM DURING NUCLEAR TRANSLOCATION IN A HORMONE-DEPENDENT MAMMARY TUMOR.

Bodwin JS, Hirayama PH, Cho-Chung YS

Lab. Pathophysiology, NCI, NIH, Bethesda, MD, 20205

Biochem Biophys Res Commun; 103(4):1349-1355 1981

Mutual inhibition of translocations from the cytoplasm to the nucleus by estrogen receptor (ER) and cyclic AMP-binding protein was examined in a cell-free system of mammary tumors. Incubation of nuclei from hormone-dependent MTW9 rat mammary tumors with cytosol activated by 5 nano (n)M 17beta-estradiol resulted in a 4-fold increase in nuclear estrogen binding activity over the control nuclei. The presence of 100 nM cyclic AMP (cAMP) in the activated cytosol inhibited nuclear uptake of ER by 50%. Incubation of the nuclei with cytosol activated by 100 nM cAMP increased nuclear cAMP binding and cAMP-dependent protein kinase activity 4-fold. The presence of 5 nM 17beta-estradiol in the activated cytosol inhibited the nuclear cAMP binding and the protein kinase activity by 50%. No competition was found between estrogen and cAMP for each other's cytoplasmic binding proteins or the nuclear acceptor sites. Data suggest that a mutual antagonism exists between the cAMP-binding protein and ER during their nuclear translocation; the mutual antagonism was dependent on concentrations of the ligands in the activated cytosol. Activated glucocorticoid receptors did not influence the nuclear uptake of either ER or cAMP-binding proteins. Studies on the structural conformation of cAMP- and estrogen binding proteins under controlled conditions of phosphorylation and dephosphorylation may elucidate the mechanism of antagonism between the receptors. (15 Refs)

MEMBRANE/ SURFACE PROTEINS

35. THE ROLE OF GLYCOCONJUGATES IN METASTATIC MELANOMA BLOOD-BORNE ARREST AND CELL SURFACE PROPERTIES.

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J Supramol Struct Cell Biochem; 17(4):325-336 1981

The role of glycoconjugates in cell-surface and blood-borne implantation (BBI) properties of murine melanoma cells (MMC) were studied. MMC of lines B16-F1 (which has low BBI potential) and B16F10 (which has high BBI potential) were exposed in vitro to tunicamycin (0.5 ug/ml for 36 hr), which prevents glycosylation of glycoproteins by inhibiting formation of lipid-linked oligosaccharide precursors. Modification of cell-surface glycoproteins was examined by measuring the decrease in binding by sialogalactoproteins of 125I-labeled Ricinus communis agglutinin I. Treatment with tunicamycin reduced protein synthesis in B16-F10 cells by 50% and reduced DNA synthesis and growth rates in both MMC strains. Tunicamycin reduced BBI in the lungs of C57BL/6 mice receiving iv injections of MMC, without an apparent shift to extrapulmonary sites. Electron microscopic examination showed that B16-F1 and B16-F10 cells treated with tunicamycin developed morphologic changes, including rounding of the cells and formation of numerous surface blebs. Treatment with tunicamycin reduced in vitro adhesion of MMC to endothelial cell monolayers, endothelial basal lamina, and polyvinyl-immobilized fibronectin. Results suggest that tunicamycin reduces experimental metastasis of murine melanoma by modifying surface glycoproteins that determine the adhesive properties of MMC. (62 Refs)

36. CHANGES IN CELL SURFACE GLYCOPROTEINS AND CARBOHYDRATE STRUCTURES DURING THE DEVELOPMENT AND DIFFERENTIATION OF HUMAN ERYTHROID CELLS.

Fukuda M, Fukuda MN

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J Supramol Struct Cell Biochem; 17(4):313-324 1981

Studies of changes in cell-surface glycoproteins (GP) and carbohydrate structures during development and maturation of human erythroid cells (HEC) are reviewed. HEC were examined by surface labeling with galactose oxidase/NaB[3H]4 (GO) or periodate/NaB[3H]4 (PI) techniques followed by digestion with endo-beta-galactosidase. Two classes of GP were detected: sialoglycoproteins labeled by PI, and band 3 and band 4.5 GP labeled by GO. Overall results indicate the following: GP of bands 3 and 4.5 are barely detectable in proerythroblasts (ProE), become evident in late erythroblast (EB) maturation, and increase towards the mature RBC stage. Glycophorins are present in ProE before Hb synthesis and increase greatly in amount during maturation. GP with mol wt of 105,000 and 95,000 are major GP in ProE and decline during maturation, being minor GP in EB. Lactosaminoglycan increases during maturation from minimal amounts in ProE; the greatest increase occurs as bands 3 and 4.5 are expressed. Molecular branching of carbohydrate structures increases greatly during maturation. In general, maturation of HEC is marked by drastic changes in surface GP and carbohydrate structures. (45 Refs)

37. IDENTIFICATION OF EXPOSED SURFACE GLYCOPROTEINS IN UNDIFFERENTIATED AND DIFFERENTIATED MOUSE N-18 NEUROBLASTOMA CELLS.

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Dept. Chemistry, Wright-Rieman Lab., Rutgers Univ., P.O. Box 939, Piscataway, NJ, 08854
Biochim Biophys Acta; 685(1):61-70 1982

A simple method is described that permitted rapid isolation of plasma membranes from mouse N-18 neuroblastoma cells. The purified plasma membranes gave a 10-fold increase in the specific activity of incorporated [3H]fucose over that of the cell homogenate. The specific activities of two other membrane markers, 5'-nucleotidase and alkaline phosphatase, increased 11-fold and 15-fold, respectively. Metabolic labeling with [3H]fucose identified a major fucosyl glycoprotein with apparent mol wt of 92,000. Three surface labeling methods together with SDS-polyacrylamide gel electrophoresis and fluorography were used to characterize and compare the surface glycoproteins of undifferentiated and differentiated N-18 cells. The galactose oxidase/NaB3H4 method labeled two major galactoproteins (Mr = 52,000, 42,000) in both undifferentiated and differentiated cells. The neuraminidase/galactose oxidase/NaB3H4 method revealed many sialylgalactoproteins. Among them, the 220-kdalton, 150-kdalton and 130-kdalton bands were at least 100% more prominently labeled in the differentiated cells whereas the 76-kdaltons and 72-kdalton bands were less prominently labeled in the differentiated cells when compared to their undiffere ntiated counterparts. The prominently iodinated protein bands in the undifferentiated cells had apparent mol wt of 130,000, 92,000, 76,000 and 72,000 as compared to 150-, 130-, 92-and 76-kdalton bands in the differentiated cells. The labeling data obtained will enable us to further study the changes of these identified surface glycoproteins, both quantitatively and topologically, during the differentiation of neuroblastoma cells. (Author abstract) (28 Refs)

38. IMMUNOCHEMICAL STUDIES OF INFECTIOUS MONONUCLEOSIS. VIII. A GLYCOPROTEIN FROM SHEEP ERYTHROCYTES WITH SIALIC ACID-DEPENDENT RECEPTOR PROPERTIES.

Fletcher MA, Caldwell KE, Latif ZA, Cayer M, Claflin A
Dept. Medicine R42, Univ. Miami Sch. Medicine, P.O. Box 016960, Miami, FL, 33101
J Immunol; 128(2):976-982 1982

A glycoprotein was solubilized from sheep erythrocyte membranes with host aqueous ethanol. The glycoprotein was purified by phosphocellulose chromatography, ethanol precipitation, lipid solvent extraction, and DEAE chromatography. In water solution the glycoprotein existed as globular aggregates with a diameter of 7.1 +-2.2 nm. In the presence of sodium dodecyl sulfate, 80% of the material exhibited a subunit mol wt app of 27,000. Approx 10% of the material had a mol wt app of only 9,000 and another 10% had a mol wt of 35,000. All three fractions were reactive with Paul-Bunnell heterophile antibody from the sera of patients with infectious mononucleosis and with Limulus polyphemus lectin. These activities were destroyed by neuraminidase treatment. Complete inhibition of the rosetting of sheep red blood cells by 4 x 10(5) human peripheral blood lymphocytes was seen at 100 to 200 ug glycoprotein/ml. Neuraminidase-treated glycoprotein was not inhibitory. Pronase-derived sialoglycopeptide was inhibitory. Most likely, the receptor for lymphocytes resides in the carbohydrate portion of the glycoprotein. By using 125I-glycoprotein, binding studies were carried out that yielded an estimate of approx 2 x 10(5) binding sites for sheep erythrocyte glycoprotein per lymphocyte. Purified glycoprotein contained 44.4% amino acid. Carbohydrate components and their molar ratios were sialic acid (1.0):galactose (1.0):N-acetylglucosamine (1.3): N-acetylgalactosamine (1.2). (Author abstract) (33 Refs)

39. CELL SURFACE MOLECULES INVOLVED IN FIBRONECTIN-MEDIATED ADHESION. A STUDY USING SPECIFIC ANTISERA.

Hughes RC, Butters TD, Aplin JD
Natl. Inst. Medical Res., The Ridgeway, Mill Hill, London NW7 1AA, England
Eur J Cell Biol; 26(1):198-207 1981

Attachment and spreading of baby hamster kidney (BHK) fibroblasts to fibronectin-coated surfaces can be inhibited by antibodies, and the (Fab)2 fragments, prepared against the cells. The antibodies reacted specifically with ricin-binding glycoproteins of the cell surface, the major com ponents having mol wt of 130-140 K. The antibodies reacted also with mouse fibroblasts (L-cells) and Chinese hamster ovary (CHO)-cells and inhibited their fibronectin-mediated adhesion to inert surfaces. Antisera raised against material isolated from the underside of BHK cells spread out on fibronectin-coated substrata using a cleavable, photolabile heterobifunctional cross-linking reagent also affected the interactions of cells with fibronectin-coated surfaces. The antibodies stained the periphery of BHK cells and L-cells in a discontinuous periodic manner and immunoprecipitated as a major component a low mol wt (approximately 47 K) glycoprotein from cell extracts. These results indicate that different specific antisera modify cell-substratum adhesion, probably by interacting with different cell surface components. The properties of multiple factors involved in the attachment and spreading of cells to suitably adhesive substrata are discussed. (Author abstract) (31 Refs)

40. TURNOVER OF PLASMA MEMBRANE PROTEINS AND GLYCOPROTEINS IN NORMAL AND REGENERATING LIVER AND MORRIS HEPATOMA 7777.

Tauber R, Reutter W
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Eur J Cell Biol; 26(1):35-42 1981

Insertion and loss of individual polypeptides and glycopolypeptides were investigated in the plasma membrane of hepatic tissue at three states of differentiation: (a) in normal liver, (b) regenerating liver and (c) in the Morris hepatoma 7777. Newly synthesized polypeptides and glycopolypeptides were inserted into the plasma membrane of all three tissues at different rates. In vivo labeling was performed with either L-[35S]methionine or L-[14C]fucose. Plasma membrane proteins were degraded heterogenously with at least two dynamically different classes in all three tissues. In normal liver three polypeptide bands, mol wt of 78 K, 94K and 165 K, were lost from the plasma membrane with an apparent half-life of less than 4 hr, whereas the bulk of plasma membrane proteins was degraded with an av half-life of 40 hr. In Morris hepatoma four polypeptide bands with mol wt of 22K, 36K 63K and 87K exhibited the same rapid degradation. Two of the short-lived polypeptides in liver (78K and 94K), and hepatoma (63K and 87K) were fucosylpolypeptides. There is evidence, that these two fucosylpolypeptides were lost from the plasma membrane by shedding into the cytosol. In normal liver rates of degradation of long-lived polypeptides correlated with the polypeptide mol wt, indicating that larger proteins may turn over at higher rates. In regenerating liver and hepatoma degradation of polypeptides of either high or low mol wt was inhibited to the same extent. It is concluded, that these readjustments in the relative rates of degradation of membrane polypeptides are a characteristic feature of proliferating hepatic tissue. (49 Refs)

41. IDENTIFICATION OF NEURAL AND ADRENAL MEDULLARY SURFACE MEMBRANE GLYCOPROTEINS RECOGNIZED BY ANTISERA TO CULTURED RAT SYMPATHETIC NEURONS AND PC12 PHEOCHROMOCYTOMA CELLS.

Lee VM, Greene LA, Shelanski ML
Dept. Neuropathology, Univ. Pennsylvania, Phila., PA, 19104
Neuroscience; 6(12):2773-2786 1981

The distribution of glycoproteins in the nervous system and recognized by antisera prepared against cultured rat sympathetic neurons and PC12 pheochromocytoma cells cultured with or without nerve growth factor (+ or-3 NGF) was examined. Antisera prepared against cultured PC12 cells and rat sympathetic neurons recognized a limited number of membrane glycoproteins. Species of glycoproteins recognized by the antisera were qualitatively similar. PC12-and PC12+ antisera immunoprecipitated the same group of fucosylated components. Components included the four recognized by antisera to rat sympathetic neurons. A close qualitative similarity between the antigenic glycoproteins present on PC12+ cells and PC12-cells and on rat sympathetic neurons was observed. NGF greatly increased the relative amount of a trypsin-sensitive, fucose- and glucosamine- containing component, termed NGF-inducible large external (NILE) glycoprotein. Results suggest that rat sympathetic neurons synthesize a material closely resembling NILE glycoprotein. (24 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

42. EFFECTS OF DENSITY AND CELLULAR AGING ON COLLAGEN SYNTHESIS AND GROWTH KINETICS IN KELOID AND NORMAL SKIN FIBROBLASTS.

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In Vitro; 18(1):79-86 1982

Cell proliferation and collagen synthesis were analyzed in high, medium, and low density keloid and normal fibroblasts and also at various times during the course of in vitro aging to expand previous findings of increased collagen synthesis in keloid compared to normal skin fibroblasts. It was found that both keloid and normal fibroblasts (less than 20 population doublings) responded similarly to high, medium, and low initial plating densities; however, sparsely plated keloid fibroblasts exhibited a loss of replicative capacity earlier in their in vitro lifespan than did sparsely plated normal skin fibroblasts. When analyzed at population doubling levels 2 to 38, collagen synthesis was elevated in keloid compared to normal skin fibroblasts but decreased at the same rate in both cell types throughout this in vitro interval. (Author abstract) (21 Refs)

43. MESSENGER RNA FOR HUMAN TISSUE PLASMINOGEN ACTIVATOR.

Opdenakker G, Weening H, Collen D, Billiau A, De Somer P
Rega Instituut, Katholieke Universiteit te Leuven, Minderbroedesstraat 10, B-3000 Leuven, Belgium
Eur J Biochem; 121(2):269-274 1982

A human melanoma cell line (Bowes), which secretes extrinsic (tissue-type) plasminogen activator, was used as a source for the preparation of mRNA for extrinsic plasminogen activator. The cells were lysed and total RNA was extracted with the phenol method. A poly(A)-rich RNA fraction was isolated by affinity chromatography on oligo(dT)-cellulose. This preparation was translated by oocytes into (a) protein(s) that had biological activity in the assay for extrinsic plasminogen activator. On sucrose gradient centrifugation the translatable fraction of the RNA migrated with a sedimentation coefficient of approx 19 S, a value compatible with the mol wt of extrinsic plasminogen activator (70,000). The translation product was characterized as being similar to or identical with authentic extrinsic plasminogen activator by the following criteria: (a) serological cross-reactivity with purified extrinsic plasminogen activator in neutralization and immunoprecipitation reactions; (b) plasminogen dependency of fibrinolytic activity and (c) apparent mol wt of 70,000 on sodium dodecyl sulfate/polyacrylamide gel electrophoresis. (Author abstract) (23 Refs)

44. PHOSPHORYLATION OF RIBOSOMAL PROTEIN S6: RELATIONSHIP TO PROTEIN SYNTHESIS IN HELA CELLS.

Nielsen PJ, Duncan R, McConkey EH
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Eur J Biochem; 120(3):523-527 1981

The time of S6 phosphorylation and several aspects of protein synthesis have been studied in suspension cultures of HeLa cells, following transfer to fresh medium and serum. The phosphorylation of S6 is not temporally correlated with changes in polypeptide initiation and elongation rates, as judged from polysome profiles. Phosphorylation of S6 can be maximal within 30 min after transfer; elongation and initiation rates increase coordinately and more slowly, becoming maximal about 6 hr after transfer, a time at which the net phosphorylation of protein S6 is greatly reduced or negligible. Recruitment of messenger RNA into polysomes is another response to fresh medium and serum; this response occurs almost as rapidly as the phosphorylation of S6. We suggest that the phosphorylation of S6 may play a role in messenger RNA recruitment. (Author abstract) (20 Refs)

45. BIOSYNTHESIS AND PROCESSING OF A HUMAN PRECURSOR COMPLEMENT PROTEIN, PRO-C3, IN A HEPATOMA-DERIVED CELL LINE.

Morris KM, Goldberger G, Colten HR, Aden DP, Knowles BB
Children's Hosp. Medical Center, Harvard Medical Sch., Boston, MA, 02115
Science; 215(4531):399-400 1982

A 180,000-dalton single-chain molecule (human pro-C3) is the precursor of the third component of human complement (C3), a disulfide-linked two-chain protein. The pro-C3 is converted by limited proteolysis to C3. The relationship between pro-C3 and C3 was established with the use of Hep G2, a cell line derived from a human hepatocellular carcinoma, which synthesizes at least 17 plasma proteins. (Author abstract) (30 Refs)

46. POSTTRANSLATIONAL MODIFICATIONS IN THE BIOSYNTHESIS OF TYPE IV COLLAGEN BY A HUMAN TUMOR CELL LINE.

Pihlajaniemi T, Myllyla R, Alitalo K, Vaheri A, Kivirikko KI
Dept. Medical Biochemistry, Univ. Oulu, Oulu, Finland
Biochemistry; 20(26):7409-7415 1981

Factors responsible for the high extent of intracellular posttranslational modifications in Type IV collagens were studied in a cultured human tumor cell line, HT-1080. These cells do not synthesize any detectable amounts of interstitial collagens but produce Type IV collagen at a high rate, corresponding to about one-third of the production of interstitial collagens by cultured human skin fibroblasts. Prolyl 4-hydroxylase activity was lower in the HT-1080 cells than in human skin fibroblasts, there being a rough correlation between this enzyme activity and the rate of 4-hydroxyproline formation in these two cell types. The differing extents of the respective modifications could largely be explained by differences in the activities of lysyl hydroxylase and the hydroxylysyl glycosyltransferases between the two cell types. No difference was found in prolyl 3-hydroxylase activity, however, even though the extent of 3-hydroxylation of proline residues was about six-fold in the Type IV collagens. In experiments where the HT-1080 cells were studied in suspension, a lag of about 100 min was found before the secretion of Type IV collagen from the cells became linear. Pulse-chase experiments in suspension indicated that all the intracellular enzyme reactions proceeded for about 40 min, presumably due to the slow triple-helix formation in Type IV collagens. This slow helix formation apparently contributed to the high extent of all the intracellular modifications but was not a major factor. (Author abstract) (45 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

47. DOES CADMIUM ADMINISTRATION CHANGE PEPTIDE ELONGATION IN RAT LIVER?

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Environ Res; 27(1):36-45 1982

Female rats received two consecutive daily injections of Cd++ (11 micromole/kg body wt) as cadmium chloride. The liver was removed 24 hr later and homogenate fractions were tested for peptide synthesis. The incorporation of [14C]phenylalanyl-tRNA into peptide and the binding of phenylalanyl-tRNA to ribosomes, ie, elongation factor 1 activity, were significantly increased in the pH 5 supernatant fraction from liver of treated rats compared to control rats. When elongation factor 1 was separated from elongation factor 2 by gel filtration, each of the factors exhibited increased peptide-synthesizing activit y in preparations from the cadmium-treated rats. (Author abstract) (29 Refs)

48. ACTION OF X-RAYS AND NEURAMINIDASE ON PINOCYTOTIC ACTIVITY AND MICROTUBULE-MICROFILAMENT SYSTEM OF EHRLICH ASCITES TUMOR CELLS IN MONOLAYER CULTURE.

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Klinik und Poliklinik fur Strahlentherapie und Rontgendiagnostik, Univ. Marburg, Robert-Koch-Str. 8a, D-3550 Marburg, W. Germany
Strahlentherapie; 157(7):468-473 1981

Monolayer cultures of Ehrlich ascites tumor cells in exponential growth phase were treated with x-rays and neuraminidase alone or in combination. A radiation dose of 2 Gy (200 rad) and higher effected a significant inhibition of DNA synthesis and cell proliferation. Neuraminidase treatment in addition to irradiation did not modify the growth-inhibitory irradiation effect. Cells pretreated for 0.5 to 1.0 hr with neuraminidase (in Earle's salt solution) and cultured thereafter (4 hr) in serum-containing growth medium exhibited an enhanced development of the microtubule-microfilament-system. Morphometric analysis of microtubules on electron-microscopic sections revealed a nearly four-fold increased number in neuraminidase treated cells while the average length of microtubules was similar to controls. Pinocytosis as measured by the ultrastructural uptake of ferritin appeared to be increased following neuraminidase treatment. A connection between neuraminic acid containing components (glycoproteins or glycolipids?) of the cell membrane or cell surface, on the one hand, and the cytoskeleton and the endocytotic process of these tumor cells, on the other hand, is suggested. (Author abstract) (25 Refs) (In GER)

49. RADIATION-INDUCED STRUCTURAL CHANGES IN HUMAN ERYTHROCYTE MEMBRANE PROTEINS REVEALED BY SODIUM DODECYL SULFATE/POLYACRYLAMIDE GEL ELECTROPHORESIS.

Todo T, Yonei S, Kato M
Lab. Radiation Biology, Faculty of Science, Kyoto Univ., Kitashirakawa, Sakyo, Kyoto 606, Japan
Radiat Res; 89(2):408-419 1982

Differences in the radiosensitivity of the major proteins of human erythrocyte membranes was investigated. It was determined that disulfide formation is a minor event and more complex reactions are involved in the radiation-induced damage to membrane proteins. Analysis of the proteins by sodium dodecyl sulfate/polyacrylamide gel electrophoresis revealed the appearance of both cross-linked aggregates and radiolytic products of spectrin. Spectrin was the most sensitive of the erythrocyte membrane proteins to x-irradiation. (34 Refs)

50. REORGANIZATION OF HELA CELL CYTOSKELETON INDUCED BY AN UNCOUPLER OF OXIDATIVE PHOSPHORYLATION.

Maro B, Bornens M
Centre de Cytologie Experimentale du CNRS, 67 rue Maurice Gunsbourg, 94200 Ivry s/Seine, France
Nature; 295(5847):334-336 1982

Effects on the cytoskeleton of impairment of mitochondrial functions induced by three metabolic inhibitors: sodium azide, which inhibits electron transport; oligomycin, which inhibits ATP synthetase; and carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP), a proton ionophore which uncouples electron transport from ATP synthesis, were studied. FCCP induced a disruption of HeLa microtubules after 1 hr. After 6 hr treatment, short microtubules were seen regrowing from the juxtanuclear microtubule-organizing centres (MTOC). FCCP had a slower effect on vimentin filaments. The filaments began to aggregate after 1 hr treatment and gathered as perinuclear bundles after 6 hr. The distribution of actin was apparently unchanged. Doses of FCCP used were non-toxic for the cells. Effects of FCCP were absent with doses below 10(-5) M and complete at 3 x 10(-5) M. Similar but weaker effects were provoked by 2,4-dinitrophenol (DNP). MTOC could be easily identified and microtubules disappeared. Loose bundles of vimentin filaments were observed around the nuclei, distributed in a different pattern from that seen in control cells. Oligomycin and sodium azide had no such effects on the cells. Results showed that the effects of FCCP are not provoked by mitochondrial ATP depletion, but could be linked to another event related to rapid dissipation of the mitochondrial electrochemical gradient. (13 Refs)

51. SELECTIVE INHIBITION BY SODIUM BUTYRATE OF GLUCOCORTICOID-INDUCED TYROSINE AMINOTRANSFERASE SYNTHESIS IN HEPATOMA TISSUE-CULTURED CELLS.

Tichonicky L, Santana-Calderon MA, Defer N, Giesen EM, Beck G, Kruh J
Institut de Pathologie et Biologie Cellulaire et Moleculaire, Centre Hospitalier Universitaire Cochin-Port Royal, 24 Rue du Faubourg-Saint-Jacques, F-75014 Paris, France
Eur J Biochem; 120(3):427-433 1981

Sodium butyrate at a 5 mM concentration prevents the induction of tyrosine aminotransferase in hepatoma culture cells, without affecting the basal level of the enzyme. This effect is reversible immediately after the removal of butyrate, or after a lag, if butyrate was present for more than 2 hr. Neither the amount of cellular RNA nor the rate of total RNA synthesis were affected by sodium butyrate. Furthermore butyrate does not inhibit protein synthesis: [35S]methionine incorporation into proteins, measured in a reticulocyte lysate system, shows no significant difference between the translation capacity of the RNAs from butyrate-treated cells and from dexamethasone-induced or uninduced cells. Nevertheless, when tyrosine aminotransferase was isolated from the translation products by its specific antiserum and analyzed by gel electrophoresis, we observed that the amount of the enzyme synthesized in the presence of RNAs from dexamethasone/butyrate-treated cells was strongly diminished relative to that synthesized in the presence of RNA from dexamethasone-induced cells. These experiments indicate that the treatm ent of the cells with butyrate decreases the activity of the specific messenger RNA for tyrosine aminotransferase to a level close to the basal level. Butyrate does not prevent the penetration of the hormone and has a limited effect on the translocation of the glucocorticoid-receptor complex to the nucleus. We therefore conclude that the effect of sodium butyrate might be inherent either to an impairment of an early step of the hormone-chromatin interaction or to an alteration of the transcription process of some specific genes. The rapidity and the complete reversibility of the effects of butyrate suggest that it does not irreversibly alter the structures which are the targets of its action. (Author abstract) (51 Refs)

DIETARY AND NUTRITIONAL EFFECTS

52. AMINO ACID AND ENERGY REQUIREMENTS FOR RAT HEPATOCYTES IN PRIMARY CULTURE.

Schwarze PE, Solheim AE, Seglen PO
Dept. Tissue Culture, Norsk Hydro's Inst. Cancer Res., Norwegian Radium Hosp., Montebello, Oslo 3, Norway
In Vitro; 18(1):43-54 1982

The amino acid and energy requirements of rat hepatocytes in suspension and early culture were investigated. Among a number of potential energy substrates tested, pyruvate (20 mM) was found to be most effective in stimulating hepatocytic protein synthesis. Amino acids stimulated protein synthesis both as energy substrates and as protein precursors. An amino acid mixture was designed to provide maximal inhibition of protein degradation as well as maximal stimulation of protein synthesis. In a defined medium containing amino acids at these concentrations, and supplemented with glucocorticoid hormone and insulin, hepatocytes could be maintained - on a collagen substratum - for at least a wk without any significant net loss of cells or cellular protein. (Author abstract) (60 Refs)

53. HISTIDINE DEFICIENCY, FOOD INTAKE AND GROWTH IN NORMAL AND WALKER 256 CARCINOSARCOMA-BEARING RATS.

Radcliffe JD, Morrison SD
IIT Res. Inst., 10 West 35th St., Chicago, IL
Nutr Cancer; 3(1):40-45 1981

Effects of isonitrogenous, isoenergetic, semisynthetic diets, including a casein-based diet, a complete free amino acid mixture simulating casein, and the same amino acid mixture minus histidine, on growth of male Sprague-Dawley rats and Walker 256 tumor-bearing rats were determined. Growth of rats on casein and free amino acid mixture diets was almost identical. Only in later stages of tumor growth after tumor-induced hypophagia had been induced were differences noted in tumor-bearing and non-tumor-bearing rats on the histidine-free diet. (19 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

54. REGULATION OF PUTRESCINE METABOLISM IN EHRLICH ASCITES CARCINOMA CELLS EXPOSED TO HYPOTONIC MEDIUM.

Kapyaho K, Janne J
Dept. Biochemistry, Univ. Helsinki, SF-00170 Helsinki 17, Finland
Biochim Biophys Acta; 714(1):93-100 1982

When exposed to hypotonic growth medium, Ehrlich ascites carcinoma cells showed a rapid stimulation of ornithine decarboxylase activity in 4 hr, followed by a rise in their putrescine content. This effect was totally abolished by addition of a slightly hypertonic concentration of sodium chloride or sucrose to the medium. The general protein synthesis was unaffected by the hypotonic treatment. The uptake of putrescine and, to a lesser extent, spermidine was enhanced, and the conversion of the radioactive putrescine into spermidine appeared partially inhibited during later stages of the hypotonic treatment. As a result, the half-life of putrescine increased from 2.8

hr under isoosmotic conditions to 7.3 hr in hypoosmotic medium. Both exogenous ([14]-putrescine-derived) and endogenous ([14C]ornithine-derived) putrescine degraded at similar rates in control and hypotonic cells, yet the putrescine taken from the medium degraded preferably to nonpolyamine products, while the putrescine synthesized in the cell was converted evenly to spermidine and to other metabolites. Adenosyl-methionine decarboxylase activity (EC 4.1.1.50), which provides the second precursor for spermidine and spermine synthesis, was distinctly inhibited in the hypotonic medium. Inhibition was likewise observed in spermidine synthase activity, while spermine synthase was marginally stimulated. It appears that the hypotonic treatment serves a special condition under which not only the formation of putrescine is enhanced dramatically but the cells also attempt to conserve the diamine by preventing its further metabolism to higher polyamines. (Author abstract) (35 Refs)

55. POLYAMINES IN MYCOPLASMAS AND IN MYCOPLASMA-INFECTED TUMOUR CELLS.

Alhonen-Hongisto L, Veijalainen P, Ek-Kommonen C, Janne J
Dept. Biochemistry, Univ. Helsinki, SF-001 70 Helsinki 17, Finland
Biochem J; 202(1):267-270 1982

Three out of four different mycoplasma strains analyzed for the polyamine contents contained relatively high concentrations of putrescine, cadaverine, spermidine and spermine. In addition to ornithine decarboxylase (EC 4.1.1.17) activity, the mycoplasmas also exhibited comparable or higher lysine decarboxylase (EC 4.1.1.18) activity fully resistant to the action of 2-difluoromethylornithine, an irreversible inhibitor of eukaryotic ornithine decarboxylase. 2-Difluoromethylornithine did not modify the polyamine pattern of actively growing mycoplasmas. Ehrlich ascites carcinoma cells and L1210 mouse leukemia cells infected with any of the four mycoplasma strains contained, in addition to putrescine, spermidine and spermine, and also easily measurable concentrations of cadaverine; the latter diamine was absent in uninfected cultures. When the infected cells were exposed to difluoromethylornithine, the accumulation of cadaverine was markedly enhanced. The modification of cellular polyamine pattern by mycoplasmas, especially in the presence of inhibitors of eukaryotic ornithine decarboxylase, could conceivably by used as an indicator of mycoplasma infection in cultured animal cells. (Author abstract) (14 Refs)

56. CYTOCHALASIN B INHIBITS POLYAMINE BIOSYNTHESIS IN HELA CELLS.

Sunkara PS, Nishioka K, Rao PN
Merrell Res. Center, 2110 E. Galbraith Road, Cincinnati, OH, 45215
Eur J Cell Biol; 26(1):154-157 1981

The potent inhibitor of cytokinesis in mammalian cells, cytochalasin B (CB), has been shown to cause the rapid inhibition of ornithine decarboxylase and S-adenosyl methionine decarboxylase activities followed by a gradual but marked decline in intracellular levels of both putrescine and spermidine. Further, the multinucleation induced by CB could be reversed to some extent by exogenous addition of polyamines to the growth medium. The results of this study suggested that polyamines might have some indirect role in the CB-induced multinucleation of mammalian cells. (Author abstract) (16 Refs)

OTHER RELATED STUDIES

57. POSSIBLE CONFORMATION OF INTERFERONS: A PREDICTION BASED ON AMINO ACID COMPOSITION AND SEQUENCE.

Zav'yalov VP, Denesyuk AI
All-Union Res. Inst. Applied Microbiology, 142200 Serpukhov, Moscow Region, USSR
Immunol Lett; 4(1):7-14 1982

Content of alpha-helices and beta-structures in interferon from fibroblast and WBC was determined by the method based on analysis of amino acid composition. Alpha-helical segments in the primary structure were localized with use of empiric and stereochemical rules. Analysis of two alternative conformations showed an anomalous distribution of charged amino acid residues in the primary structure of the interferons. (13 Refs)

58. BACTERIAL EXPRESSION OF AN ENZYMATICALLY ACTIVE PROTEIN ENCODED BY RSV SRC GENE.

McGrath JP, Levinson AD
Dept. Molecular Biology, Genentech, Incorporated, 460 Point San Bruno Blvd., South San Francisco, CA, 94080
Nature; 295(5848):423-425 1982

A bacterial plasmid was constructed which is capable of expressing in Escherichia coli a protein (p60) closely related in size and immunological properties to a 60,000-molecular wt (Mr) phosphoprotein (pp60src). The p60 protein appeared to be the translational product of the src gene under the control of the trp promoter. Total cellular proteins were analyzed in E coli transformed with recombinant vector pBR322, pHGH207-1, or pSRCex16. No overall change in the protein profile occurred in cells transformed with pBR322. In cells transformed with pHGH207-1, a prominent protein of 22,000 Mr appeared. Only in pSRCex16-transformed cells, was a 60,000 Mr p60 protein induced; p60 appeared selectively during late growth phase. When induced, p60 represented approx 5% of total bacterial protein. Further characterization of the protein was performed. Protein kinase (PK) activity was assayed in recombinant E coli strain harboring either pBR322 or the p60 expression plasmid (pSRCex16). PK activity was present only in bacteria expressing p60; PK activity, when expressed, was tyrosine-specific. The finding of a tyrosine-specific PK activity in a highly purified bacterially-derived preparation of p60 lended support to the conclusion that this enzymatic activity represents an intrinsic property of the molecule. (35 Refs)

59. ANTI-F MET-LEU-PHE: SIMILARITIES IN FINE SPECIFICITY WITH THE FORMYL PEPTIDE CHEMOTAXIS RECEPTOR OF THE NEUTROPHIL.

Marasco WA, Showell HJ, Freer RJ, Becker EL
Dept. Pathology, Univ. Michigan, Box M045, Pathology Building, 1335 East Catherine St., Ann Arbor, MI, 48109
J Immunol; 128(2):956-962 1982

We have prepared antisera in both rabbits and rats against f Met-Leu-Phe conjugated to a variety of carrier proteins. Over 40 peptides with widely varying reactivity for the neutrophil formylpeptide receptor have been tested for their ability to bind to rabbit antibody raised against fMLP10-BSA. Structure-activity studies of peptides structurally related to f Met-Leu-Phe demonstrate that the N-formyl group is mandatory for maximum antibody binding activity. Methionine in position 1 and phenylalanine in position 3 are found to confer maximum binding activity. Stereoselectivity of the antibody-combining site also has been demonstrated. Comparison of the ability of the peptides to bind to the antibody receptor with their reactivity for the neutrophil has demonstrated a strong correlation in the rank order of reactivity of the numerous synthetic peptides; for the alphaNH2-acyl group, $r = 0.94$; for position 1, $r = 0.90$; for position 2, $r = 0.97$; and for position 3, $r = 0.78$. This strong correlation is seen across species lines with both rabbit and rat antibodies. Significant differences, however, in the specificity of the antibody and neutrophil receptors are seen at the carboxyl terminus of phenylalanine, and beyond the phenylalanine ring, $r = 0.29$. In addition, bacterial chemotactic factor-enriched butanol extracts from Escherichia coli culture filtrates can also bind to anti-F Met-Leu-Phe, affording additional evidence for the similarity in the structure of the bacterial chemotactic factor to the synthetic chemotactic peptides. (Author abstract) (34 Refs)

60. DNA SEQUENCE OF A GENE ENCODING A BALB/C MOUSE LD TRANSPLANTATION ANTIGEN.

Moore KW, Sher BT, Sun YH, Eakle KA, Hood L
(c/o Hood) Div. Biology, California Inst. Technology, Pasedena, CA, 91125
Science; 215(4533):679-682 1982

The sequence of a gene, denoted 27.5 encoding a transplantation antigen for the BALB/c mouse has been determined. Gene transfer studies and comparison of the translated sequence with the partial amino acid sequence of the Ld transplantation antigen establish that gene 27.5 encodes an Ld polypeptide. A comparison of the gene 27.5 sequence with several complementary DNA sequences suggests that the BALB/c mouse may contain a number of closely related L-like genes. Gene 27.5 has eight exons that correlate with the structural domains of the transplantation antigen. (Author abstract) (22 Refs)

61. PARTIAL CHARACTERIZATION OF THE MRNA THAT CODES FOR ENKEPHALINS IN BOVINE ADRENAL MEDULLA AND HUMAN PHEOCHROMOCYTOMA.

Comb M, Herbert E, Crea R
Dept. Chemistry, Univ. Oregon, Eugene, OR, 97403
Proc Natl Acad Sci USA; 79(2):360-364 1982

The compounds [Met]enkephalin and [Leu]enkephalin are derived from a protein in bovine adrenal medulla that contains multiple copies of [Met]enkephalin. Here we characterize proenkephalin mRNA from bovine and human tissue by use of an oligodeoxynucleotide pentadecamer probe complementary to codons for Tyr-Gly-Gly-Phe-Met ([Met]enkephalin). This probe hybridizes specifically to a species of poly(A)-RNA from adrenal medulla and human pheochromocytoma, (1,400-1,450 bases), and also to [Met]enkephalin-containing pro-opiomelanocortin mRNAs from bovine pituitary (1,200 bases) and from mouse pituitary tumor cells (1,100 bases). A cloned cDNA probe (144 bases) complementary to the region of pro-opiomelanocortin mRNA that codes for lipotropin does not hybridize to the RNA from bovine adrenal medulla, demonstrating that the latter RNA is not pro-opiomelanocortin mRNA. The pentadecamer probe was extended to make cDNA with reverse transcriptase after hybridizing it to adrenal poly(A)-RNA. The sequence of an extended cDNA, 6[illegible] bases in length, was found to correspond exactly to that expected from the amino acid sequence of peptide E (a bovine adrenal peptide containing [Met]- and [Leu]enkephalin sequences). This cDNA also forms a specific hybrid with the RNA from bovine adrenal and human pheochromocytoma, confirming that these species of RNA are pro-enkephalin mRNA. (Author abstract) (28 Refs)

62. APPARENT METHIONINE AUXOTROPHY OF SOME TUMOUR CELL LINES MAY BE LINKED TO IMPAIRED AMINO ACID TRANSPORT.

Tisdale MJ
Dept. Pharmacy, Univ. Aston in Birmingham, Gosta Green, Birmingham B4 7ET, England
Eur J Cancer Clin Oncol; 17(12):1323-1329 1981

An investigation of the L-methionine requirement of five cell lines in vitro shows an increased dependence on extracellular L-methionine in the order: human embryonic bladder fibroblasts (HE), human bladder carcinoma (EJ), mouse lymphoma (TLX5), mouse bladder carcinoma (MB) and Walker rat carcinoma (W.256). This order correlates with the initial velocity of transport of this amino acid and the steady state concentrations achieved. The order of L-methionine requirement is the same as the order of the ability of these cell lines to survive and grow in alpha L-methionine-depleted medium containing only L-homocysteine. The tumor cell lines all have a decreased maximal initial rate of L-methionine transport (Vmax) and a lower Km value than two normal cells investigated (hepatocytes and fibroblasts). Kinetic studies permit the identification of two transport systems for methionine in W 256 and TLX5 and only one for the other cell lines. The initial rate of transport of L-lysine in these cell lines follows the same order as for L-methionine. Studies using L-[methyl-3H]-methionine labeled cells show that, in addition to a defective transport system for methionine in W.256 and MB, the rate of loss of label from nucleic acids and proteins is also higher for these two cell lines. These results suggest that the apparent methionine auxotrophy of some tumor cell lines may be due to an impaired capacity to concentrate methionine and a higher turnover rate. (Author abstract) (20 Refs)

63. ENDOTHELIAL CELL DAMAGE AFTER GAMMA-IRRADIATION IN VITRO: IMPAIRED UPTAKE OF ALPHA-AMINOISOBUTYRIC ACID.

Kwock L, Doublas WH, Lin PS, Baur WE, Fanburg BL
Dept. Radiology, Univ. North Carolina Sch. Medicine, Chapel Hill, NC, 27514
Am Rev Respir Dis; 125(1):95-99 1982

The effects of gamma-irradiation with a cesium-137 source were assessed on endothelial cells isolated from bovine pulmonary arteries and maintained in culture. The radiobiologic parameters that characterize the dose-response survival curve for these cells, expressed as mean +- SD were found to be $n = 1.6 +-0.2$, $Dq = 71 +-13$ and $DO = 161 +-35$ rad, indicating a relatively low capacity of these cells to accumulate or repair radiation damage. Increasing doses of radiation led to decreasing uptake by endothelial cells of [14C]2-aminoisobutyric acid (AIB), a Na+ dependent and nonmetabolizable amino acid. A dose of 500 rad, which caused marked inhibition of cell survival, resulted in a 45% decrease in AIB uptake at 6 hr and a 69% decrease in uptake at 24 hr after irradiation. No morphologic abnormality was noted in these cells by light microscopy at 24 hr after this dose of radiation. Bovine pulmonary artery fibroblasts, on the other hand, showed no significant impairment in uptake of AIB 24 hr after exposure to doses of radiation as high as 5,000 rad. The uptake by endothelial cells of [14C]1-aminocyclopentane-1-carboxylic acid, an amino acid transported by a Na+ independent process, was not influenced by 5,000 rad of radiation. Our studies show that endothelial cells are sensitive to radiation and that impaired Na+ dependent uptake of AIB represents an early event in radiation damage to the endothelial cell. (Author abstract) (23 Refs)

64. DIRECT PHOTOAFFINITY LABELING OF AN ALLOSTERIC SITE ON SUBUNIT PROTEIN M1 OF MOUSE RIBONUCLEOTIDE REDUCTASE BY DTTP.

Eriksson S, Caras IW, Martin DW
Dept. Biochemistry, Karolinska Inst., Stockholm, Sweden
Proc Natl Acad Sci USA; 79(1):81-85 1982

The protein M1 subunit of ribonucleotide reductase contains at least two allosteric nucleotide binding sites that control the capacity of the enzyme to reduce ribonucleotides to the deoxyribonucleotides required for DNA synthesis. Direct photoaffinity labeling of partially purified protein M1 from mouse T-lymphoma (S49) cells was observed after UV irradiation in the presence of dTTP at 0 C. The relative molar incorporation of nucleotide per subunit was 4-8%. Competition experiments showed that the dTTP was bound to an allosteric domain genetically and kinetically defined as the substrate specificity site of the enzyme. An altered protein M1 isolated from a thymidine-resistant mutant cell line showed significantly decreased photoincorporation of dTTP, consistent with the fact that its CDP reductase activity is resistant to feedback inhibition by dTTP. Specific photolabeling of several other proteins with pyrimidine and purine nucleotides was also found, indicating the general usefulness of direct photoaffinity labeling in the study of enzymes involved in nucleotide and nucleic acid metabolism. (Author abstract) (11 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Aden DP 45
Ait-Ahmed O 2
Alhonen-Hongist 55
Alitalo K 27
Alitalo K 46
Anderson NG 26
Aplin JD 39
Bach JF 33
Bagdasarian A 29
Barrach HJ 21
Baumbach L 3
Baur WE 63
Beck G 51
Becker EL 59
Bernfield M 20
Bichler E 32
Billiau A 43
Blair HE 22
Bodwin JS 34
Bohn H 24
Bornens M 50
Bricas E 33
Brouet JC 23
Bushkin Y 19
Butters TD 39
Caldwell KE 38
Caras IW 64
Casnellie JE 7
Cayer M 38
Charm S 22
Chen KY 37
Chiba M 18
Cho-Chung YS 34
Claflin A 38
Cohen IK 42
Cole RD 1
Collen D 43
Colman RW 29
Colten HR 45
Comb M 61
Corrales JJ 31
Crea R 61
Dardenne M 33
Daume E 24
David G 20
Daxenbichler G 32
De Somer P 43
Defer N 51
Dellagi K 23
Denesyuk AI 57
Djondjurov L 5
Doublas WH 63
Duncan R 44
Eakle KA 60
Edwards JJ 26
Ek B 8
Ek-Kommonen C 55
Eriksson S 64
Fanburg BL 63
Farrelly JG 12
Fletcher MA 38
Freer RJ 59
Fukuda M 36
Fukuda MN 36
Galdun J 42
Gartemann A 6
Gastinel LN 33
Giesen EM 51
Glasgow JE 29
Goldberger G 45
Greene LA 41
Guevara J 26
Hammerling U 19
Harrison ML 7
Heldin CH 8
Hellstrom KE 7
Herbert E 61
Hess F 48
Hilz H 6
Hirayama PH 34
Hoisaeter PA 31
Hood L 60
Hughes RC 39
Inaba N 24
Iqbal MP 11
Irimura T 35
Irwin D 15
Ishiguro I 9
Iype PT 12
Izant JG 16
Izant JG 17
Janne J 54
Janne J 55
Jimbow K 18
Jimbow M 18
Kapyaho K 54
Karr JP 31
Kato M 49
Kern HF 48
Kikutani H 19
Kirdani RY 31
Kivirikko KI 46
Knowles BB 45
Krebs EG 7
Kruh J 51
Kwock L 63
Latif ZA 38
Lee VM 41
Leivo I 27
Lenger K 4
Levinson AD 58
Lin PS 63
Luftig RB 16
MacLaughlin DT 25
Mangeat P 2
Marasco WA 59
Marashi F 3
Marchis-Mouren 2
Maro B 50
Martin DW 64
Marvaldi J 2
Mascardo RN 16
Matsuzawa T 9
McConkey EH 44
McCoy BJ 42
McGrath JP 58
McIntosh JR 17
McMahon JB 12
Merker HJ 21
Meshulam DH 22
Minowada J 22
Moore KW 60
Morgat JL 33
Morris KM 45
Morrison SD 53
Moss B 13
Murphy GP 31
Myllyla R 46
Nelson WJ 10
Nicholls DM 47
Nicolson GL 35
Nielsen PJ 44
Nishioka K 56
Opdenakker G 43
Orr FW 28
Paul N 48
Paulin D 23
Pehrson JR 1
Perreau J 23
Pfab R 48
Pihlajaniemi T 46
Pike LJ 7
Piontek GE 28
Pleau JM 33
Radcliffe JD 53
Rakhra GS 47
Rao PN 56
Renk T 24
Reutter W 40
Richardson GS 25
Rickles R 3
Rinehart CA 37
Risteli J 30
Risteli L 27
Risteli L 30
Rocklin RE 22
Rose IA 14
Rothenberg SP 11
Rubartelli A 19
Sandberg AA 31
Santana-Caldero 51
Sasaki A 33
Schachtschabel 48
Schwarze PE 52
Schwoch G 6
Seglen PO 52
Shelanski ML 41
Sher BT 60
Sherline P 16
Showell HJ 59
Sierra F 3
Sitia R 19
Sobue M 9
Solheim AE 52
Stavnezer J 19
Stein GS 3
Stein JL 3
Sugimoto N 9

Sun YH 60
Sunkara PS 56
Sylvan PE 25
Tauber R 40
Tichonicky L 51
Timpl R 27
Tisdale MJ 62
Todo T 49
Tollaksen SL 26
Traub P 10
Vaheri A 27
Vaheri A 46
Varani J 28
Veijalainen P 55
Venkatesan S 13
Von Eschenbach 26
Ward PA 28
Warms VB 14
Wartiovaara J 27
Wass JA 28
Wasteson A 8
Weatherbee JA 16
Weatherbee JA 17
Weber W 6
Weening H 43
Weihing RR 16
Westermark B 8
Wielckens K 6
Wong BL 22
Yancheva N 5
Yonei S 49
Zav'yalov VP 57

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In this issue:

- **NUCLEOPROTEINS** **1-3**
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** **4-9**
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** **10-22**
- **BINDING/ RECEPTOR PROTEINS** **23-26**
- **MEMBRANE/ SURFACE PROTEINS** **27-36**
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** **37-39**
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** **40-49**
- **DIETARY AND NUTRITIONAL EFFECTS** **50-52**
- **POLYAMINES - SYNTHESIS AND METABOLISM** **53-56**
- **OTHER RELATED STUDIES** **57-65**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service • National Institutes of Health • National Cancer Institute

NUCLEOPROTEINS

1. NOREPINEPHRINE AND NERVE GROWTH FACTOR: SIMILAR PROTEINS PHOSPHORYLATED IN THE NUCLEI OF TARGET CELLS.

Hashimoto S, Guroff G
Lab. Developmental Neurobiology, Natl. Inst. Child Health and Human Development, NIH, Bethesda, MD, 20205
Biochem Biophys Res Commun; 104(4):1477-1483 1982

Treatment of C6 glioma cells (ATCC CCL 107) with the beta-adrenergic agonist isoproterenol, in the presence of (32P) inorganic phosphate, increased radioactivity in two nonhistone nuclear proteins. Comparison of radioautograms of these nonhistone fractions with those of nuclei of superior cervical ganglia treated with nerve growth factor show strong similarity of the proteins. (18 Refs)

2. REGULATION OF DNA LIGASE ACTIVITY BY POLY(ADP-RIBOSE).

Creissen D, Shall S
Cell and Molecular Biology Lab., Univ. Sussex, Brighton, Sussex BN1 9QG, England
Nature; 296(5854):271-272 1982

Nuclear ADP-ribosyl transferase (ADPRT) catalyzes transfer of ADP-ribose from NAD to chromatin proteins to form mono, oligo- and poly(ADP-ribose) modified chromatin proteins. Various other studies are reviewed which suggest that (ADP-ribose)n biosynthesis is necessary for effective repair of some types of DNA damage. The question remains as to which step in DNA repair requires ADPRT activity. Experiments are described which support the hypothesis that ADP-ribosylation activates DNA ligase activity. It is suggested that ADPRT has a general function in the control of the breaking and rejoining of DNA. (46 Refs)

3. DNA LIGASE AND DNASE ACTIVITIES IN MOUSE ERYTHROLEUKEMIA CELLS DURING DIMETHYL SULFOXIDE-INDUCED DIFFERENTIATION.

Scher BM, Scher W, Robinson A, Waxman S
Div. Medical Oncology, Mount Sinai Sch. Medicine, 1 Gustave L. Levy Place, New York, NY, 10029
Cancer Res; 42(4):1300-1306 1982

DNA ligase and DNase levels were measured in cell-free extracts from untreated mouse erythroleukemia (MEL) cells and from cells treated with dimethyl sulfoxide (Me2SO) to induce erythroid differentiation. The DNase activity present in the extracts was sensitive to inhibition by G-actin and was, therefore, presumed to be DNase I. When the MEL cells were induced to differentiate by culturing in the presence of 1.8% Me2SO for 3 or 4 days, the apparent activity of DNA ligase decreased to approx 12% of the value in untreated MEL cells. In contrast, the apparent DNase I activity of the extracts from Me2SO-treated cells increased over that in extracts from untreated cells by a factor of 2. The activity of acid phosphatase, a lysosomal enzyme, remained unchanged. When strain DR-10, a mutant of the MEL cells which does not undergo Me2SO-induced differentiation, was treated with Me2SO, the DNA ligase and DNase activities of extracts from these cells remained unchanged as compared to extracts from untreated DR-10 cells. Therefore, the marked decrease in the level of DNA ligase activity appeared to be related to the process of differentiation in the Me2SO-treated MEL cells. (Author abstract) (39 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

4. ARCHITECTURE AND POLYPEPTIDE COMPOSITION OF HELA CYTOSKELETONS. MODIFICATION OF CYTOARCHITECTURAL POLYPEPTIDES DURING MITOSIS.

Bravo R, Small JV, Fey SJ, Larsen PM, Celis JE
(c/o Celis) Div. Biostructural Chemistry, Dept. Chemistry, Aarhus Univ., DK-8000 Aarhus C, Denmark
J Mol Biol; 154(1):121-143 1982

Substrate-attached asynchronous HeLa cells were extracted with Triton X-100 and analyzed by electron microscopy and two-dimensional gel electrophoresis. Such Triton cytoskeletons showed actin filament bundles, microtubules, intermediate filaments, and actin networks in the substrate-associated lamellae, and contained around 90 polypeptides (48 basic, 42 acidic; 52% of total actin, 99% of vimentin, 41% of alpha-actinin and 30% of beta-tubulin). Cytoskeletons produced by further extraction in high and low salt buffers (L-H-L) showed only intermediate filaments, the nucleus and residual actin, and contained a total of 19 polypeptides (13 acidic, 6 basic). Of these, 12 corresponded to abundant acidic proteins in the 47,000 to 70,000 Mr region as determined by staining with Coomassie blue and labeling with a mixture of 14C-labelled amino acids. Using L-H-L extracted cytoplasts, and employing an actin depolymerizing protein from slime molds, seven abundant acidic IEF polypeptides were shown to be present in these intermediate filament-enriched, substrate-attached cytoplast cytoskeletons. These polypeptides (L-H-L cytoplast polypeptides) corresponded to vimentin (IEF 26, 54,000 Mr) and six polypeptides (IEF 12, 68,000 Mr; IEF 24, 56,000 Mr; IEF 31, 50,000 Mr; IEF 35, 49,000 Mr; IEF 36, 48,500 Mr and IEF 46, 43,500 Mr) not previously reported as present in cytoskeletons. Peptide analysis showed that these were not related as products of modification or proteolysis. Labeling of mitotic and interphase cells with [35S]methionine followed by one-dimensional peptide map analysis showed that IEF 24, 26 (vimentin), 31 and 36 are preferentially modified during mitosis. These modifications correspond to phosphorylations of IEF 26 (vimentin) and 31, and to an unknown type for IEF 24. IEF 36 is phosphorylated in interphase to yield IEF 37, and the latter is further phosphorylated in mitosis. These results suggest that modification of the L-H-L cytoplast polypeptides may be important in the reorganization of cytoskeletal elements that takes place during cell division. (Author abstract) (51 Refs)

5. ISOLATION OF PRIMARY CULTURES FROM POST-MORTEM SAMPLES OF HUMAN BRAIN.

West GM, Vaughan PF, Freshney RI, McNamee HB, Graham DI
Dept. Biochemistry, Univ. Glasgow, Glasgow G12 8QQ, Scotland
Biochem Soc Trans; 10(1):53-54 1982

Various marker enzymes (cyclic nucleotide phosphohydrolase, glycerol phosphate dehydrogenase, and glutamine synthetase) were assayed in rat C6 glioma cells and compared with primary cultures derived from human brain. Four cell lines studied (G-ATA, G-CCM, G-RTA and IJK) were grown from human gliomas; two (G-CCM and IJK) are glial fibrillary acidic protein-positive. Seven samples of fetal brain were also studied along with cultures from normal adult brain (which are glial fibrillary acidic protein-negative). (3 Refs)

6. KERATIN-LIKE PROTEINS IN NORMAL AND NEOPLASTIC CELLS OF HUMAN AND RAT MAMMARY GLAND AS REVEALED BY IMMUNOFLUORESCENCE MICROSCOPY.

Krepler R, Denk H, Weirich E, Schmid E, Franke WW
Div. Gastroenterologic Pathology and Molecular Pathology, Dept. Pathology, Univ. Vienna Sch. Medicine, Spitalgasse 4, A-1090 Vienna, Austria
Differentiation; 20(3):242-252 1981

Normal and neoplastic human breast tissue as well as lactating and nonlactating rat mammary glands and 7,12-dimethylbenz(a)-anthracene-induced mammary adenocarcinomas of rat, were examined by indirect immunofluorescence microscopy using guinea pig antibodies to human and bovine epidermal prekeratin and to cytokeratin polypeptide D from mouse hepatocytes. In normal mammary glands of both species, the antibodies raised against human and bovine epidermal prekeratins strongly stained ductal and myoepithelial cells, whereas antibodies to hepatic cytokeratin D revealed, in addition, fibrillar staining in cells of the alveolus-like terminal lobular units and in milk secreting cells of the rat. Our results show that all types of neoplastic and non-neoplastic epithelial cells of mammary gland of both species contain filaments of the cytokeratin type identifiable by immunologic reaction, if antibodies are used that recognize a broad range of epidermal and nonepidermal cytokeratins. Such broad range antibodies to keratin-like proteins provide adequate tools to identify and characterize neoplastic and non-neoplastic epithelial cells and to eliminate false negative immunocytochemical findings in tumor diagnosis. Our observation that in the same human carcinoma two cell types can be distinguished by their reaction with two different antibodies to cytokeratins from epidermis and liver, respectively, indicates that the cells of a given carcinoma can differ in their cytoskeletal composition, thus presenting further criteria for diagnostic differentiation. (48 Refs)

7. IMMUNOLOGICAL DETECTION OF SPHINGOMYELIN-CARRYING PROTEIN IN TUMORS AND IN EMBRYONAL RAT LIVER.

Diatlovitskaia EV, Sinitsyna EV, Timofeeva NG, Iakimenko EF, Kuprina NI, Bergel'son LD
M. M. Shemiakin Inst. Bioorganic Chemistry, Moscow, USSR
Biokhimiia; 47(1):62-65 1982

The presence of sphingomyelin-carrying protein (SCP) was studied in the cytosol of hepatoma 27, sarcoma 45, Jensen sarcoma, nephroma RA, and in the normal, regenerating, and embryonal rat liver. SCP was assayed by double immunodiffusion technic. SCP was detected in the postmicrosomal supernatant of all tumors tested. SCP was also present in the embryonal liver. SCP was not detected in the normal and regenerating liver, or in the blood of tumor-bearing rats. (16 Refs) (In RUS)

8. CHARACTERIZATION OF IMMUNE TYPE INTERFERON (IFNGAMMA)-INDUCED CYTOPLASMIC PROTEI N KINASE ACTIVITY IN MOUSE L CELLS.

Ohtsuki K, Torres BA, Johnson HM
Dept. Microbiology, Univ. Texas Medical Branch, Galveston, TX, 77550
Biochem Biophys Res Commun; 104(2):422-429 1981

Mouse immune interferon (IFN-gamma) induced double-stranded RNA-dependent protein kinase activity in the cytoplasmic fraction of mouse L cells, measured by histone phosphorylation with (gamma-32P)ATP. The protein kinase activity was separated by column chromatography into three fractions of 100,000, 70,000, and 70,000 dalton mol wt. The fractions differed in Mg and Ca requirements, cyclic AMP activation, N-ethylmaleimide inhibition effect, and substrate requirements. (15 Refs)

9. ABNORMAL NEUTROPHIL MYELOPEROXIDASE FROM A PATIENT WITH CHRONIC MYELOCYTIC LEUKEMIA.

Atkin CL, Andersen MR, Eyre HJ
Dept. Internal Medicine, Univ. Utah Sch. Medicine, Salt Lake City, UT, 84132
Arch Biochem Biophys; 214(1):284-292 1982

Examination of subunits of purified neutrophil myeloperoxidase from a patient with chronic myelocytic leukemia revealed that compared with normal enzyme, one 55,000-dalton alpha subunit was replaced by a 39,000-dalton peptide with a different amino-terminal sequence, a mixture of smaller peptides, and a heme derivative. Myeloperoxidase from the leukemic neutrophils appeared to have been partially degraded in vivo by lysosomal proteases. (43 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

10. NEUTROPHIL MIGRATION INHIBITION FACTOR FROM T LYMPHOCYTES (NIF-T): PARTIAL PURIFICATION BY ANTIBODY AFFINITY CHROMATOGRAPHY AND FURTHER CHARACTERIZATION.

Weisbart RH, Lusis AJ, Kacena A, Spolter L, Eggena P, Golde DW
Dept. Medicine (111S), Veterans Admin. Medical Center, 16111 Plummer St., Sepulveda, CA, 91343
Clin Immunol Immunopathol; 22(3):408-418 1982

Neutrophil migration inhibition factor from T lymphocytes (NIF-T) is a newly recognized glycoprotein that is distinguished from other migration inhibition factors by its mol wt, isoelectric point, and resistance to inactivation by diisopropylfluorophosphate (DFP). NIF-T was purified approx 1,700-fold by antibody affinity chromatography. Two major peaks of NIF-T activity corresponding to mol wt of about 50,000 and 25,000 were resolved by gel filtration chromatography. The predominant mol wt species was approx 25,000 as determined by gel filtration chromatography in the presence of urea and by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The larger (50,000) component probably represents a dimer or aggregate of the smaller mol wt component. NIF-T was shown to have an isoelectric point of approx pH 4.6. Treatment with bacterial neuraminidase shifted the isoelectric point to about pH 4.8, suggesting the presence of sialic acid. (Author abstract) (14 Refs)

11. LYMPHOKINES: THEIR ROLE IN LYMPHOCYTE RESPONSES. PROPERTIES OF INTERLEUKIN 1.

Oppenheim JJ, Stadler BM, Siraganian RP, Mage M, Mathieson B
Lab. Microbiology and Immunology, Natl. Inst. Dental Res., NCI, NIH, Bethesda, MD, 20205
Fed Proc; 41(2):257-262 1982

Interleukin 1, or IL 1, otherwise known as lymphocyte-activating factor, is a macrophage-derived 12,000-to 15,000-dalton polypeptide. Isoelectric focusing of human IL 1 reveals three peaks at pI's of 5.2, 6.0, and 6.9, respectively. IL 1 can be depleted of lymphocyte-derived IL 2 by SP-Sephadex chromatography. IL 1 augments the mitogenic response of PNA- Lyt 1 thymocytes, and promotes thymocyte helper functions and B cell antibody production. IL 1 induces stable E rosette formation and the production of lymphokines such as T cell growth factor (IL 2) by peripheral T lymphocytes. Others have shown that IL 1 or closely related factors also stimulate hypothalamic cells to induce fever; induce in vitro fibroblast growth, prostaglandin, and collagenase production; and stimulate hepatocytes to produce acute phase proteins such as serum amyloid A. Murine epidermal cells also produce a 15,000-dalton factor that is mitogenic for thymocytes and may be similar to IL 1. We have recently hybridized spleen cells from mice sensitized with partially purified human IL 1 with a myeloma cell line. Clones have been isolated that produce supernatants that partially inhibit the thymocyte proliferative response to IL 1 but not the T cell growth factor activity of IL 2. Should these hybridoma products prove to be monoclonal anti-IL 1 antibodies, they will facilitate the further purification and characterization of IL 1. (Author abstract) (43 Refs)

12. ENDO- AND ECTOCERVICAL HUMAN UTERINE EPITHELIAL CELLS DISTINGUISHED BY FIBRONECTIN PRODUCTION AND KERATINIZATION IN CULTURE.

Alitalo K, Halila H, Vesterinen E, Vaheri A
Dept. Virology, Univ. Helsinki, Haartmaninkatu 3, 00290 Helsinki 29, Finland
Cancer Res; 42(3):1142-1146 1982

Human ecto- and endocervical uterine epithelia grow in culture as stratifying and nonstratifying cell layers, patterns reflecting the differentiation of the corresponding cells in vivo. We found that both cultures synthesized and secreted fibronectin but very little other known glycoproteins of basal laminae, laminin, and Type IV collagen. Fibronectin production was observed only in the lining marginal cells of the expanding periphery of ectocervical islands, but differentiating and keratinizing cells had lost fibronectin. Most cells in endocervical epithelial cultures grew as a monolayer and produced fibronectin. After stratification of the ectocervical cells, the cytokeratin polypeptide pattern switched to the large-mol-wt type. In endocervical cultures, the low-mol-wt keratin polypeptides persisted. Fibronectin production thus appears to be linked to the state of differentiation of the epithelial cells, and fibronectin may function in the attachment of the basal cells to underlying basal lamina matrix. (Author abstract) (37 Refs)

13. BASEMENT MEMBRANE OF THE UTERINE CERVIX: IMMUNOFLUORESCENCE CHARACTERISTICS OF THE COLLAGEN COMPONENT IN NORMAL OR ATYPICAL EPITHELIUM AND INVASIVE CARCINOMA.

Frappart L, Berger G, Grimaud JA, Chevalier M, Bremond A, Rochet Y, Feroldi J
Laboratoire d'Anatomie Pathologique, U.E.R. Grange-Blanche 8, Avenue Rockefeller, 69373 Lyon Cedex 2, France
Gynecol Oncol; 13(1):58-66 1982

Frozen sections of the uterine cervix were processed by an indirect immunofluorescence technique using specific antisera against Type I, III, and IV collagens (raised in rabbits). A continuous basement membrane (BM) was selectively stained using antibodies against Type IV collagens beneath both squamous and columnar epithelia. In the case of atypical epithelium, the appearance of BM beneath the epithelia remains unchanged. In contrast, with invasive carcinomas, a more or less continuous band of unequal thickness, whose reactivity in the presence of antibodies to Type IV collagen remains weak or moderate, is observed around the lobules of neoplastic cells. Thus, the unimpaired character of the basement membrane cannot be considered as the major criterion, to distinguish carcinoma in situ from invasive carcinoma of the uterine cervix. (Author abstract) (27 Refs)

14. ENHANCED SYNTHESIS OF BASEMENT MEMBRANE PROTEINS DURING THE DIFFERENTIATION OF RAT MAMMARY TUMOUR EPITHELIAL CELLS INTO MYOEPITHELIAL-LIKE CELLS IN VITRO.

Warburton MJ, Ferns SA, Rudland PS
Ludwig Inst. Cancer Res. (London Branch), Royal Marsden Hosp., Sutton, Surrey, SM2 5PX, England
Exp Cell Res; 137(2):373-380 1982

Rama 25, an epithelial cell line obtained from a dimethylbenzanthracene-induced rat mammary tumor differentiates spontaneously in culture forming elongated myoepithelial-like cells. The elongated cells form multilayered ridge structures from which cultures of elongated cells, relatively uncontaminated by epithelial cells, can be obtained. By using immunofluorescence techniques, both the elongated cells and the cells in ridges, but not undifferentiated Rama 25 cells, have been demonstrated to synthesize three basement membrane proteins, laminin, type IV collagen, and fibronectin. The identity of these basement membrane proteins has been confirmed by immunoprecipitation. These proteins appear to be located in a fibrillar extracellular matrix. We suggest that the ability to synthesize basement membrane proteins by mammary epithelial cells in vitro on plastic is a characteristic of myoepithelial-like cells. (Author abstract) (20 Refs)

15. CHANGES IN REGIONAL KERATIN POLYPEPTIDE PATTERNS DURING PHORBOL ESTER-MEDIATED REVERSIBLE AND PERMANENTLY SUSTAINED HYPERPLASIA OF MOUSE EPIDERMIS.

Schweizer J, Winter H
Inst. Experimental Pathology, German Cancer Res. Center, Im Neuenheimer Feld 280, 6900 Heidelberg, W. Germany
Cancer Res; 42(4):1517-1529 1982

The keratin polypeptide pattern of newborn mouse back, ear, and tail epidermis is uniform and consists of eight individual proteins with a mol wt range of 46,000 to 67,000. In the adult animal, the keratin patterns of the corresponding body sites are both different from the neonatal pattern and different among themselves. This postnatal specialization of the local program of keratin polypeptide synthesis cannot be related to distinct morphological criteria. We have analyzed the behavior of these regional keratinization phenotypes in the adult mouse under the influence of a reversible or a permanently sustained hyperplasia. Independent of the type of hyperplasiogen used to induce the epidermal trauma, the initial phase of a reversible hyperplasia is characterized by only marginal morphological alterations, maintenance of the normal one-dimensional keratin pattern, but pronounced changes in the charge properties of distinct keratin polypeptides, indicating disturbances of the secondary phosphorylation-dephosphorylation process. During the acute phase of reversible hyperplasia, the local keratinization phenotypes are quantitatively altered due to the enhanced expression of high-mol wt keratin polypeptides. However, the charge properties of the newly synthesized proteins appear normal. A comparison of both the sequential morphological alterations during acute hyperplasia and the temporally corresponding changes in the keratin patterns allows an assignment of the site of synthesis of distinct polypeptides within the epidermis, which is in line with findings of other laboratories. Long-term hyperplasia (2 wk to 2 mo of regular treatment) is not morphologically different from max reversible hyperplasia. However, it induces both quantitative and qualitative alterations of the local keratinization phenotypes and causes a reversal of the postnatal divergence of keratin synthesis by generally restoring the neonatal keratin pattern in all three adult epithelia investigated. (46 Refs)

16. A NON-COLLAGENOUS GLYCOPROTEIN FROM ELASTIC TISSUE ACTS AS SUBSTRATUM FOR GROWTH OF CELLS IN VITRO.

Knox P, Wells P, Serafini-Fracassini A
Dept. Biochemistry, St. George's Hosp. Medical Sch., Cranmer Terrace, London SW17 0RE, England
Nature; 295(5850):614-615 1982

In vivo, cells are in intimate contact with an extracellular matrix, one of the components of which is collagen; the adhesion of cells to collagen in vitro has been extensively studied. In several connective tissue matrices, collagen fibers are replaced to a variable extent by elastin fibers which, under the electron microscope, appear to be composed of an elastin core surrounded by a network of fibrils approx 11 nm in diameter, usually referred to as the microfibrillin component. These fibrils have been implicated, on morphological grounds, in the biosynthesis of elastin fibers. A glycoprotein referred to as structural glycoprotein (SCP), which has a mol wt (Mr) or 34,000 and aggregates to form cylindrical tactoids having a uniform diameter of 11 nm, has been isolated from bovine ligamentum nuchae. SGP was found to have amine oxidase activity, thus it seems to be functionally related to other peptidylysine oxidases involved in the biosynthesis of the elastin crosslinks. Here we show that SGP acts as a substratum for fibroblasts and other cell types with fibronectin mediating the attachment and spreading of the cells. These results suggest that SGP may have a role in wound healing, morphogenetic movement and development of elastic tissues. (Author abstract) (16 Refs)

17. SECRETION OF A THIOL PROTEINASE FROM MOUSE MAMMARY CARCINOMAS AND ITS CHARACTERIZATION.

Recklies AD, Mort JS, Poole AR
Joint Diseases Lab., Shriners Hosp. Crippled Children, Montreal, Quebec H3G 1A6, Canada
Cancer Res; 42(3):1026-1032 1982

Spontaneous mammary tumors from C3H/HeJ mice and transplants established from mammary tumors were investigated for their capacity to secrete thiol-dependent proteinase activity in organ culture explants. More activity was detected in culture media from spontaneous tumors than from transplanted spontaneous tumors. The accumulation of thiol proteinase in the culture medium was inhibited by cycloheximide, hydrocortisone, and aldosterone, but not by estradiol or the peptide hormones insulin or prolactin. The thiol proteinase is similar in enzymic properties to lysosomal cathepsin B, but its physical properties are different. It is stable to alkaline pH, has a larger molecular size on gel filtration (relative MW 39,000), and shows a different isoenzyme pattern to liver cathepsin B on analytical isoelectric focusing. The characteristics of this thiol proteinase are very similar to an enzyme secreted from malignant human breast tumors. (Author abstract) (39 Refs)

18. PRODUCTION OF HUMAN ALPHA1-ANTICHYMOTRYPSIN-LIKE PROTEIN BY A HUMAN MALIGNANT MELANOMA TRANSPLANTED INTO NUDE MICE.

Kondo Y, Ohsawa N
Ludwig Inst. Cancer Res., Royal Melbourne Hosp., Post Office, Victoria 3050, Australia
Cancer Res; 42(4):1549-1554 1982

A tissue culture line of a human malignant melanoma, SEKI, induced cachexia in nude mice (BALB/c-nu/nu). During the investigation of the cause of the cachexia, the melanoma was found to produce a protein immunologically identical to human alpha1-antichymotrypsin (alpha1-Ach). Tissues of the SEKI melanoma contained the protein immunologically equivalent to 0.29 -0.11 (SD) mg of human alpha1-Ach per g of wet tissue, while the other six human malignant tumors transplanted into nude mice did not contain a detectable amount of it. In the serum of nude mice bearing the melanoma, this protein appeared soon after the tumor growth occurred and gradually increased up to the level equivalent to 5 mg of human alpha1-Ach per dl. Removal of the tumor resulted in a rapid decrease of the protein in the serum to an undetectable level within 1 day. This protein was never detected in the serum of nude mice bearing the other 27 human malignant tumors or controls. Purification of this protein was carried out by the column chromatography using DE-52, Blue-Sepharose, and SP-Sephadex. The elution patterns were the same as those of alpha1-Ach in human serum, and the mol wt of the protein was estimated as 69,000 by Sephadex G-100 column chromatography and 65,000 by polyacrylamide gel electrophoresis with sodium dodecyl sulfate. This purified protein, however, did not exhibit inhibitory activity against chymotrypsin. These results show that this melanoma produced a protein immunologically identical and physicochemically very similar to human alpha1-Ach. This melanoma-nude mouse system may provide a useful model for investigating the synthesis of human alpha1-Ach and analysis of its physiological roles. (Author abstract) (20 Refs)

19. S-100 PROTEIN IN SOFT-TISSUE TUMORS DERIVED FROM SCHWANN CELLS AND MELANOCYTES.

Stefansson K, Wollmann R, Jerkovic M
(c/o Wollmann) Dept. Pathology, Univ. Chicago, 950 East 59th St., Chicago, IL, 60637
Am J Pathol; 106(2):261-268 1982

In soft tissues outside the central nervous system, S-100 protein is found normally only in Schwann cells. Using the peroxidase-antiperoxidase immunohistochemical method S-100 was also found in tumors derived from Schwann cells and melanocytes, including neurofibromas, neurilemomas, granular cell myoblastomas, cutaneous nevi, and malignant melanomas. S-100 was not detected in malignant Schwannomas, neuroblastomas, oat cell carcinomas, medullary carcinomas of the thyroid, paragangliomas, or meningiomas. S-100 was also absent from neoplasms of soft tissues not usually considered to arise from cells of neural crest origin. S-100 appears to be a useful marker for identifying neoplasms derived from Schwann cells and melanocytes. (Author abstract) (40 Refs)

20. INFLUENCE OF PROSTATIC SECRETION PROTEIN ON UPTAKE OF ANDROGEN-RECEPTOR COMPLEX IN PROSTATIC CELL NUCLEI.

Pousette A, Bjork P, Carlstrom K, Forsgren B, Hogberg B, Gustafsson JA

Dept. Medical Nutrition, Karolinska Institutet, S-104 01 Stockholm, Sweden

Prostate; 2(1):23-33 1981

The modulating effect of prostatic secretion protein (PSP) on the nuclear uptake of the androgen-receptor complex in the prostatic cell was investigated in vitro and the effects of estramustine on the cellular concentration of PSP were studied. PSP could be purified in sufficient quantities for further in vitro studies by chromatography on Concanavalin A. Addition of PSP decreased the amount of [3H]-R1881-receptor complex taken up by the prostatic cell nuclei by 10% to about 3% and decreased the retention of the [3H]-R1881-receptor complex on DNA cellulose from approx 25% of total androgen-receptor complex to about 3%. Castration and treatment with estradiol-17beta-valerate (2 mg/wk sc) led to drastic reductions in prostate wt and amounts of PSP/mg of total cytosolic protein. Administration of estramustine (1 mg/day im) resulted in a reduction of prostatic wt, but did not affect the amount of PSP/mg total cytosolic protein. Modulation of the concentration of PSP may be suitable for control of the effects of androgens on the prostate in a tissue-specific manner. Estramustine affects the prostate in a manner different from that of estrogens or castration. (21 Refs)

21. PURIFICATION AND PARTIAL CHARACTERIZATION OF A PROTEIN FROM CANCER ASCITES FLUID WHICH STIMULATES THE RESORPTION OF BONE EXPLANTS IN VITRO.

Nimberg RB, Humphries DE, Lloyd WS, Wells H, Schmid K

Dept. Periodontology, Henry M. Goldman Sch. Graduate Dentistry, Boston, MA, 02118

J Biol Chem; 257(5):2477-2482 1982

A protein capable of stimulating bone resorption in vitro has been purified approximately 1,250-fold from cancer ascites fluid. Purification was accomplished employing successive fractionation with ammonium sulfate, ion exchange, and Cibacron blue affinity chromatography, isoelectric focusing, and selective adsorption on hydroxylapatite. The bone-resorptive protein obtained by this procedure appeared homogeneous in polyacrylamide gels at pH 9.5, migrating with the mobility of an alpha2-globulin, and in sodium dodecyl sulfate polyacrylamide gels from which an apparent mol wt of 43,000 was calculated. The amino acid composition of the bone-resorptive protein distinguished itself by the absence of methionine and by its relatively high content of glycine (17%) and proline (11%). Furthermore, the protein possesses a single NH2-terminal amino acid residue (glycine). The ascites protein was found to contain 19% carbohydrate by weight including a high content of sialic acid (15 residues/mol) as compared to the other sugars (27 residues/mol). As to its biological properties, the homogeneous ascites glycoprotein proved to be as potent as parathyroid hormone in its ability to stimulate bone resorption in vitro. (Author abstract) (31 Refs)

22. PRODUCTION AND DISTRIBUTION OF ENTACTIN AND GP-2 IN M1536-B3 CELLS.

Bender BL, Carlin B, Jaffe R, Temple T, Chung AE
Dept. Pathology, Presbyterian-Univ. Hosp., DeSoto at O'Hara St., Pittsburgh, PA, 15213
Exp Cell Res; 137(2):415-425 1982

The endodermally differentiated mouse embryonal carcinoma cell line M1536-B3 produces the basement membrane-associated glycoproteins entactin and GP-2 in cell culture. Immunological techniques coupled with light and electron microscopy were used to study the intracellular distribution and fate of these molecules. The two proteins were localized in cisternae of rough endoplasmic reticulum and in discrete membrane-bound vesicles. There was no evidence for their presence in the Golgi apparatus. The membrane-bound vesicles appeared to fuse with the plasma membrane and thereby transfer their contents to discrete foci on the cell surface. These foci became apparent as cell-to-cell contact was made and were prominent at cell-cell contact sites. They eventually coalesced into continuous densely stained extracellular bands of amorphous material. These bands formed a honeycomb of spaces lined by cells. It appeared that production of the extracellular matrix was probably stimulated by cell-cell contact, and that cell density rather than age of the cell cultures dictated the pattern of extracellular distribution. These results support the hypothesis that these molecules are involved in cell adhesion and multicellular organization. (Author abstract) (23 Refs)

BINDING/ RECEPTOR PROTEINS

23. 1,25-DIHYDROXYVITAMIN D3 RECEPTORS IN HUMAN EPITHELIAL CANCER CELL LINES.

Colston K, Colston MJ, Fieldsteel AH, Feldman D
(c/o Feldman) Div. Endocrinology, S-005, Stanford Univ. Sch. Medicine, Stanford, CA, 94305
Cancer Res; 42(3):856-859 1982

Specific, high-affinity cytosolic receptors for 1,25-dihydroxyvitamin D3 have been demonstrated in five human cancer cell lines. The cell lines were derived from tumors of breast, lung, cervix, and melanotic and amelanotic melanomas. Binding affinity (Kd) of the receptors for 1,25-dihydroxyvitamin D3 were all approx 0.2 nM, and receptor content ranged from 21 to 174 fmol/mg cytosol protein. The receptors from all five cell lines sedimented at 3.2S on sucrose density gradients and exhibited preferential affinity for 1,25-dihydroxyvitamin D3 compared to other vitamin D metabolites. (Author abstract) (28 Refs)

24. PURIFICATION AND PARTIAL CHARACTERIZATION OF CELLULAR RETINOL-BINDING PROTEIN FROM HUMAN LIVER.

Ong DE
Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
Cancer Res; 42(3):1033-1037 1982

Cellular retinol-binding protein (CRBP) has been purified to homogeneity from normal human liver. The procedures in the purification involved primarily gel filtration and ion exchange chromatography, resulting in a 3,000-fold purification with greater than 40% yield. The protein is a single:polypeptide chain with mol wt of 14,800. The protein binds retinol in a manner which considerably alters its spectrum from that observed in organic solution. Many of the properties of human CRBP including mol wt, amino acid composition, and spectrum of bound retinol are similar to those observed previously for rat CRBP. The availability of pure human CRBP should aid in elucidating its role in the action of retinol and also in more easily monitoring the considerable changes in level of this protein reported in some human cancers. (Author abstract) (27 Refs)

25. PRESENCE OF CELLULAR RETINOL AND RETINOIC ACID-BINDING PROTEINS IN EPIDERMOID CARCINOMA OF THE ORAL CAVITY AND OROPHARYNX.

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Dept. Biochemistry, Vanderbilt Univ., Nashville, TN, 37232

Cancer; 49(7):1409-1412 1982

Epidermoid carcinomas of the oral cavity and oropharynx from six patients were examined for the presence and amount of cellular retinol (CRBP) and cellular retinoic acid-binding (CRABP) proteins. In all cases adjacent, grossly normal tissue was similarly examined. For each example CRBP levels were significantly higher in tumor tissue compared to adjacent tissue. In four cases CRABP was significantly higher. This is of interest because retinol, retinoic acid and their analogs have been shown to inhibit the development of various epithelial tumors, and this inhibition is possibly mediated by these binding proteins. (Author abstract) (28 Refs)

26. ANDROPHILIC PROTEINS IN THE HUMAN PROSTATE.

Shimazaki J, Ito H, Kodama T, Nozumi K, Maruoka M, Wakisaka M, Naito H, Miyauchi T, Matsumura T, Sato R

Dept. Urology, Sch. Medicine, Chiba Univ., Chiba 280, Japan

Prostate; (Suppl1):9-17 1981

Androphilic proteins in prostate tissues of prostatic cancer patients were compared with those from patients with benign hypertrophic prostate and 'normal' patients with bladder cancer in terms of binding of tritiated androgens. Nuclear extracts from benign hypertrophic prostate showed high affinity binding for 3H-dihydrotestosterone (DHT), suggesting that a part of the androphilic proteins in cytosols of human prostate is an androgen receptor. A large part of the components binding with R1881 in cytosol were shown to be due to binding sites for progestins; nuclear extracts from human prostate contained a protein that bound to R1881, suggesting that R1881 binding comprises two parts, an androgen receptor and a progestin-binding protein. The latter is not a progestin receptor because nuclear extracts did not bind to R5020. Tissues from 'normal' and benign hypertrophic prostates showed almost identical dissociation constants for DHT, R1881, and R5020. The max binding sites of these ligands were very similar among the tissues, except that the max binding sites of DHT were higher in 'normal' than in pathological prostates. (10 Refs)

MEMBRANE/ SURFACE PROTEINS

27. THE ASPARAGINE-LINKED SUGAR CHAINS OF PLASMA MEMBRANE GLYCOPROTEINS OF K-562 HUMAN LEUKAEMIC CELLS: A COMPARATIVE STUDY WITH HUMAN ERYTHROCYTES.

Yoshima H, Shiraishi N, Matsumoto A, Maeda S, Sugiyama T, Kobata A

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J Biochem (Tokyo); 91(1):233-246 1982

The paper electrophoretic pattern of the oligosaccharides released from the plasma membranes of K-562 cells by hydrazinolysis was quite different from that of human erythrocyte membranes. Bio-Gel P-4 column chromatography in combination with sequential exoglycosidase digestion of the neutral oligosaccharide fractions revealed that all those from K-562 cells are of the high mannose type, while those from erythrocytes are of large complex type structures. Studies of the acidic oligosaccharides indicated that none of those obtained from K-562 cells contained the beta-N-acetylglucosamine residue linked at the C-4 position of the beta-mannosyl residue of the trimannosyl core, which occurs in most of the asparagine-linked sugar chains of human erythrocytes. This indicates that the glucosaminyltransferase that forms the GlcNAcbeta1 to 4Manbeta1 to 4 group has not been expressed in K-562 cells. (Author abstract) (43 Refs)

28. TERATOCARCINOMA CELL ADHESION: IDENTIFICATION OF A CELL-SURFACE PROTEIN INVOLVED IN CALCIUM-DEPENDENT CELL AGGREGATION.

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Cell; 28(2):217-224 1982

Teratocarcinoma cells have a Ca-dependent cell-cell adhesion site (t-CDS) that is unique in being inactivated with trypsin in the absence of Ca but not in the presence of Ca. Fab fragments of antibodies raised against teratocarcinoma F9 cells dissociated by treatment with trypsin and calcium (anti-TC-F9) inhibit the aggregation of teratocarcinoma cells mediated by t-CDS. This inhibitory effect of Fab is removed when anti-TC-F9 is absorbed with F9 cells treated with trypsin and calcium (TC-F9), but not when it is absorbed with F9 cells treated with trypsin and EGTA (TE-F9). Comparisons of cell-surface antigens reactive to anti-TC-F9 in TC-F9 cells with those in TE-F9 cells reveal that only one component, with an approx mol wt of 140,000 (p140), is detected specifically on the surface of TC-F9 cells. When TC-F9 cells are retrypsinized in the absence of Ca, a substance with an approx mol wt of 34,000 (p34) is released that can neutralize the aggregation-inhibitory effect of the Fab. This p34 interferes with the immunoprecipitation of p140 with anti-TC-F9, suggesting that p34 is a tryptic fragment of p140. Anti-TC-F9 Fab causes the dissociation of the monolayers of teratocarcinoma cells. This effect is removed by absorption of the Fab with p34 as well as with TC-F9 cells, but not with TE-F9 cells. These results suggest that p140 is essential for the function of t-CDS, and that this is an actual cell-adhesion molecule active in the establishment of monolayers of teratocarcinoma cells. (Author abstract) (37 Refs)

29. SURFACE GLYCOPROTEINS OF DIVIDING AND NON-DIVIDING THYMOCYTES.

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Dept. Biochemistry, St George's Hosp. Medical Sch., London, SW17, ORE, England
Cell Biol Int Rep; 6(2):189-195 1982

The surface glycoproteins labeling of rat large, mainly dividing thymocytes and their small, non-dividing, progeny, by reduction with sodium [3H]borohydride after various oxidative treatments of the cells, is described. Results showed little difference between the two cell types except in the band at 51,000 mol wt, and even after no oxidative pretreatment of the cells a labeled band was obtained at 53,000 mol wt with a minor component at 47,000 mo wt. Pretreatment with galactose oxidase induced weak labeling of other proteins including bands at 150,000, 100,000, and 25,000 mol wt. (17 Refs)

30. ELECTROPHORETIC ANALYSIS OF RADIOLABELED CELL SURFACE PROTEINS AND GLYCOPROTEINS OF SOME HUMAN HEMOPOIETIC CELL LINES.

Chorvath B, Duraj J, Stockbauer P, Babusikova O
Cancer Res. Inst., Slovak Acad. Sciences, 812 32 Bratislava, Czechoslovakia
Neoplasma; 28(6):625-632 1981

Cell surface proteins and glycoproteins of several human lymphoblastoid- and neoplastic hemopoietic cell lines were radiolabeled by lactoperoxidase catalyzed iodination and by sodium periodate/tritiated sodium borohydride. Electrophoretic patterns of radiolabeled proteins and glycoproteins obtained by electrophoresis under denaturing conditions (SDS-PAGE) were essentially similar for all examined lymphoblastoid cell lines, with a characteristic group of major radiolabeled glycoproteins gp44 and gp24,31. Characteristic, individually different and distinguishable patterns of radiolabeled proteins were observed in different neoplastic hemopoietic cell lines: T-leukemia cell line MOLT 3, erythroleukemic cell line K562, pre-B cell leukemia line NALM 6, and a promyelocytic leukemia cell line HL-60. Cell surface proteins of HL-60 promyelocytic leukemia cell line displayed some similarities to those of another myeloid leukemia cell line ML 3. Examined Burkitt lymphoma cell lines were similar to lymphoblastoid cell lines with some minor differences. Glycoprotein gp44, markedly labeled on lymphoblastoid cell lines, was absent on Burkitt lymphoma cell line Daudi. Electrophoretic patterns of cell surface proteins of blast cells from a few patients with leukemia examined simultaneously with the cell lines are described and discussed. (Author abstract) (28 Refs)

31. IMMUNOPRECIPITATION OF MEMBRANE PROTEINS OF CULTURED HUMAN SARCOMA CELLS.

Grofova M, Forchhammer J, Lizonova A, Popovic M
Cancer Res. Inst., Slovak Acad. Sciences, 812 32 Bratislava, Czechoslovakia
Neoplasma; 28(6):633-645 1981

Human sarcoma associated antigens (HSAA) have previously been identified by indirect immune fluorescence in human sarcoma cells in culture using sera from patients bearing different types of sarcoma. To further characterize these HSAA, surface proteins of cultured cells were labeled with 125Iodine, [3H]-glucosamine and [35S]-methionine and solubilized. After immunoprecipitation labeled proteins were detected in immune complexes by SDS polyacrylamide gel electrophoresis and autoradiography, which allowed comparison with antigens described by other groups. A surface protein (Mr 96,000) was precipitated with sera from sarcoma bearing patients, and two glycoproteins (Mr 115,000 and 85,000) were preferentially precipitated with antisera from rabbits immunized with membranes from two human sarcoma cell lines. At least two of these proteins were found in each of five human sarcoma cell lines studied (U-4SS, U-3930S, U-20S, B-5GT and B-6FS). None of the proteins were precipitated with three human control sera, and only occasionally a faint band was observed in immunoprecipitates from control cells (B-25F, B-41B, B-42FC, U-2S, and U-393S) with the immune sera. These proteins are probably some of the antigens responsible for the immune fluorescence observed in determination of HSAA. However, purification of the proteins and competition experiments are needed before this can be finally established. (Author abstract) (26 Refs)

32. MODULATION OF PROTEIN PHOSPHORYLATION IN HUMAN COLON ADENOCARCINOMA CELL MEMBRANE PREPARATIONS BY EPIDERMAL GROWTH FACTOR IN VITRO.

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Int J Cancer; 29(3):277-282 1982

Incubation of membranes prepared from the human colon adenocarcinoma cell line LoVo in vitro with [gamma-32P]ATP demonstrated numerous components whose phosphorylation was stimulated several fold by epidermal growth factor (EGF). One major component of Mr 170 K, which was either undetectable or very weakly phosphorylated in the absence of EGF, was primarily affected by exposure to EGF. The phosphorylation of the 170 K Mr membrane component required the presence of Mn2; Mg2 was ineffective. Although the phosphorylation of many LoVo membrane components was significantly modified by cAMP or dibutyryl-cAMP, none of these cyclic nucleotides appeared to be involved in the phosphorylation of the 170 K membrane component in the presence or absence of EGF. The phosphorylation system of the LoVo membranes efficiently utilized [gamma-32P]GTP in both the basal and EGF-stimulated reactions. All the membrane components phosphorylated in the absence or presence of EGF, except a band comigrating with the tracking dye front, were digested by trypsin. The possible glycoprotein nature of the 170 K dalton phosphoprotein was indicated by the fact that the 170 K dalton band comigrated with a periodic acid-Schiff base-stained band. These findings suggest that one of the biochemical steps in the mechanism of action of EGF in LoVo cells is enhanced phosphorylation of several membrane proteins, especially that of a glycoprotein of Mr 170 K, by a membrane-bound cyclic nucleotide independent protein kinase. (Author abstract) (23 Refs)

33. 5'-NUCLEOTIDASE ACTIVITY IN PERMANENT HUMAN LYMPHOID CELL LINES: IMPLICATION FOR CELL PROLIFERATION AND AGING IN VITRO.

Sun AS, Holland JS, Ohnuma T, Slankard-Chahinian M
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Biochim Biophys Acta; 714(3):530-535 1982

The 5'-Nucleotidase of 11 human lymphoid cell lines was measured. These cell lines were homogeneous B, T and Null cells, had an unlimited lifespan in vitro, and were subcultivated from leukemic cells of patients with Burkitt lymphoma, the blastic phase of chronic myelocytic leukemia, or acute lymphoblastic leukemia. 5'-Nucleotidase activities in normal human lymphocytes and in human fibroblasts (VA-13 and IMR-90) could be determined at a cellular protein concentration as low as 0.025 and 0.007 mg/ml, respectively. In all the 11 lymphoid cell lines, including 8 B-cell lines (RPMI 8422, B46M, RPMI 1788, DAUDI, HRIK, B411-4, B85 and DND-3-9A), 2 T-cell lines (MOLT 3 and RPMI 8402) and 1 Null cell line (NALM-1) 5'-nucleotidase was undetectable with the protein concentration range from 0.033 to 8.543 mg/ml. Previously 5'-nucleotidase activity was found to increase 10-, 6-and 20-fold in normal human embryonic lung (WI-38 and IMR-90 cells) and chick embryo fibroblasts, respectively, from a young rapidly proliferative to a senescent non-proliferative stage. These data demonstrate that the large increase in 5'-nucleotidase activity occurs concomitantly with the in vitro senescence of these normal cell lines. The present study suggests that this large increase in 5'-nucleotidase activity during cell aging is absent in these permanent lymphoid cell lines. The undetectable 5'-nucleotidase activity may be a biochemical characteristic of these homogeneous B, T and Null cells originating from the aforesaid leukemias. The implications of these results for cell proliferation and aging are discussed. (Author abstract) (28 Refs)

34. GENETIC CHARACTERIZATION OF A POLYMORPHIC MURINE CELL-SURFACE GLYCOPROTEIN.

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Dept. Cancer Biology, Salk Inst., Post Office Box 85800, San Diego, CA, 92138
Immunogenetics; 15(3):313-320 1982

As described in the preceding paper, monoclonal antibodies have been raised by immunization of rats with mouse hematopoietic cells which detect a major cell-surface glycoprotein (Mr = 9 5,000) of mouse bone-marrow cells of the granulocytic series. While most of the monoclonal antibodies detect this molecule on bone-marrow and spleen cells of all mouse strains, two antibodies recognize alternative allelic forms of the molecule. One alloantigen is expressed in all the remaining inbred strains examined. The alloantigens are codominantly expressed on the cells of F1 mice. Backcrosses of DBA/2 and C57BL/6 with F1 mice (B6D2F1) confirmed that a single genetic locus is involved in the expression of the two antigenic forms and demonstrated linkage to Ly-m11 which has previously been mapped to mouse chromosome 2. These genetic mapping experiments and the biochemical properties of the glycoprotein suggested that it might be identical to a glycoprotein first identified on murine fibroblasts by Hughes and August and designated Pgp-1. This has been firmly established by exchange of monoclonal antibody reagents and sequential immunoprecipitations. (Author abstract) (16 Refs)

35. CELLULAR ADHESION TO GROWTH SURFACES: A POSSIBLE RÔLE FOR LOW MOLECULAR WEIGHT SURFACE COMPONENTS.

Wildridge M, Sherbet GV
Cancer Res. Unit, Dept. Clinical Biochemistry and Metabolic Medicine, Univ. Newcastle upon Tyne, Newcastle upon Tyne 4LP, England
Exp Cell Biol; 50(1):27-33 1982

Cell lines initiated from a fibrocystic hyperplasia and a carcinoma of the breast and an established human fetal lung fibroblast line, MRC5, were grown in culture. The surface proteins of the cells were iodinated in cell monolayer, and in suspension following harvesting from culture flasks using EDTA. Data are presented which show that cells labeled in suspension show reduced incorporation of 125I into surface proteins of high mol wt (greater than 100,000 daltons) and considerably increased incorporation into proteins with mol wt less than 100,000 daltons. These changes are discussed in relation to the possible release of surface material by the action of EDTA. It is also suggested, on the basis of the current findings, that the detachment of cells may expose low mol wt components possibly involved in the attachment of the cells to the substratum. (Author abstract) (18 Refs)

36. LOSS OF FIBRONECTIN AMONG THE SELECTIVE SURFACE PROTEIN CHANGES ASSOCIATED WITH P-FORMALDEHYDE PROMOTED MEMBRANE VESICULATION.

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Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 1010-A, Venezuela
Biochem Biophys Res Commun; 104(2):844-850 1981

Radioiodination followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to detect proteins in vesicle fractions released when cultured cells were subjected to 25 milli(m)M p-formaldehyde and 2 mM dithiothreitol (DTT). Surface iodination of 'normal' WIRL-3 cells, 'transformed' R72/3 cells, normal rat kidney fibroblasts, and Rat-1 normal fibroblasts revealed upon electrophoresis a major 220 kilodalton (kd) fibronectin-like component. Vesiculation induced by the p-formaldehyde/DTT treatment decreased the component in all but the Rat-1 cells, and increased the lower mol wt fibronectin components, whether iodination took place before or after chemical vesiculation induction. In Rat-1 cells the vesiculation treatment caused a decrease in a surface-associated 140 kd component with an increase in a 130 kd component in the vesicular fraction. The release and selective degradation of cell surface proteins upon chemical vesiculation may involve surface protease activation. (12 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

37. COMPARISON OF PROTEIN SYNTHESIS BY LYMPHOCYTES FROM BLOOD OF DONORS AND PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

Morozova EO, Blinov MN
Inst. Hematology and Blood Transfusion, Leningrad, USSR
Vopr Onkol; 27(12):28-33 1981

An assay of lymphocyte ribosomes in blood of donors and patients with chronic lymphocytic leukemia established the normal level of ribosomes in 11 cases and half that value in 6 cases of leukemia. The decreased level of ribosomes in cells was largely registered at the terminal stage of the disease. The 14C-leucine uptake by leukemic lymphocytes was reduced two- to three-fold while the relative level of 14C-leucyl-tRNA in diseased and normal cells was identical. The rate of lymphocyte protein synthesis was lowered in a wide range (2-8-fold), in most of patients (7 out of 8). The rate of protein synthesis (radioactivity of acid insoluble fraction per mg of ribosomes) was half the normal value and constant in leukemic lymphocytes. An important role of ribosome level in protein biosynthesis regulation in the course of disease progression is suggested. (Author abstract) (25 Refs) (In RUS)

38. PROLACTIN-DEFICIENT VARIANTS OF GH3 RAT PITUITARY TUMOR CELLS: LINKED EXPRESSION OF PROLACTIN AND ANOTHER HORMONALLY RESPONSIVE PROTEIN IN GH3 CELLS.

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Dept. Molecular and Population Genetics, Univ. Georgia, Athens, GA, 30602

Mol Cell Biol; 2(2):179-189 1982

Expression of prolactin (PL) and the hormonally responsive protein p21 was studied in variants of GH3 rat pituitary tumor cells. GH3 cells in vitro produced approx equal amounts of PL and growth hormone (GH). After exposure to ethylmethanesulfonate (EMS: 300 ug/ml) for 24 hr, survival of GH3 cells was 20-30%. Cultures of 3 of 12 surviving mutants (LP), produced PL in greatly reduced amounts (1.0-2.5% of the amount produced by wild-type GH3 cells). GH production by LP cells varied only approx twofold from GH production by wild-type cells. Radiolabeling experiments showed that reduced secretion of PL resulted from reduced synthesis of PL. Reduced synthesis of PL was repressible by dexamethasone. Reduction in PL synthesis was accompanied by a reduction in p21, which, like PL, is repressible by glucocorticoids (GC). Production of other GC-repressible proteins was not reduced in LP cells. PL production varied in different LP. In the B2 variant reversion to normal PL production occurred at a rate of approx 20%. In the B3 variant reversions occurred at a frequency of 0.5%. Reversion to normal PL production was accompanied by a return to normal amounts of p21, indicating that PL and p21 are closely linked. GH production was phenotypically stable in LP and in GH3 cells not exposed to EMS. Results suggest that EMS, an alkylating agent, promoted an epigenetic change in gene expression. (37 Refs)

39. ORIGIN OF BRONCHIOLOALVEOLAR CARCINOMA AND PERIPHERAL BRONCHIAL ADENOCARCINOMA.

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Cancer; 49(5):881-887 1982

The morphologic distribution of newly synthesized glycoproteins in a bronchioloalveolar carcinoma and in a peripheral bronchial adenocarcinoma with areas of bronchioloalveolar differentiation was determined and compared with bronchiolar epithelium and Type II alveolar cells. Tissues were maintained in vitro, and autoradiography was used to reveal locations of glycoproteins metabolically labeled with 3H-glucosamine. Both tumors displayed glycoprotein secretory activity as most incorporated radioactivity was rapidly released from cells. Silver grains were associated with cytoplasmic granules and mucin-like secretions in the bronchioloalveolar carcinoma and granules plus unidentified secretions in the bronchial adenocarcinoma. Clara cells exhibited an association of newly synthesized glycoproteins with cytoplasmic granules similar to those found in the tumors. In bronchiolar ciliated epithelium, as in bronchial ciliated cells and central bronchogenic adenocarcinomas, glycoproteins accumulated over apical cell surfaces and were not rapidly secreted. Type II cells incorporated label into secreted contents of cytosomal inclusions and unidentified cytoplasmic material. The observations suggest that a bronchioloalveolar carcinoma and a peripheral bronchogenic adenocarcinoma are distinct from common bronchogenic adenocarcinomas and are derived from secretory cells that resemble bronchiolar Clara cells. (Author abstract) (20 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

40. THE EFFECT OF BENZO(A)PYRENE ON DNA SYNTHESIS AND DNA POLYMERASE ACTIVITY OF RAT LIVER MITOCHONDRIA.

Salazar I, Tarrago-Litvak L, Gil L, Litvak S
Departamento de Bioquimica, Facultad de Medicina, Universidad de Chile, Casilla 6671, Santiago 7, Chile
FEBS Lett; 138(1):45-49 1982

DNA synthesis in liver mitochondria of benzo(a)pyrene (BP: 40 mg/kg)-treated rats was higher than in mitochondria from untreated rats, both at different protein concentrations and over different incubation times, but the solubilized mitochondrial DNA polymerase activity was higher in the control preparation. Dideoxythymidine triphosphate (ddTTP) inhibited DNA synthesis in whole mitochondrial preparations, with a much more profound effect in mitochondria from untreated rats. Detergent treatment showed that the difference between treated and untreated mitochondria was not due to BP effect on organelle membrane permeability. Ethidium bromide produced a polymerase inhibiting effect similar to that of ddTTP, but aphidicolin did not. (21 Refs)

41. INHIBITORY EFFECTS OF METHYL GLYOXAL ON DNA, RNA AND PROTEIN SYNTHESIS IN CULTURED GUINEA PIG KERATOCYTES.

White JS, Rees KR
Dept. Biochemical Pathology, Sch. Medicine, Univ. Coll. London, University St., London WC1E 6JJ, England
Chem Biol Interact; 38(3):339-347 1982

The inhibitory action of methyl glyoxal upon protein, RNA and DNA synthesis in guinea pig keratocytes has been investigated. Methyl glyoxal directly inhibits each of these processes. Inhibition of protein synthesis results from an action upon initiation of translation. Methyl glyoxal induces polysome degradation which is prevented by pretreatment with cycloheximide. Further analysis of translation indicates that elongation and termination are not affected. Inhibition of ribosomal RNA synthesis results from inhibition of maturation. Methyl glyoxal was found to inhibit the appearance of 28S rRNA in the cytoplasm from pulse-labeled precursors. The significance of these results is discussed in relation to cell injury by ketals. (Author abstract) (21 Refs)

42. SELECTIVE EARLY LOSS OF POLYPEPTIDES IN LIVER MICROSOMES OF CCL4-TREATED RATS.

Noguchi T, Fong KL, Lai EK, Olson L, McCay PB
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Biochem Pharmacol; 31(5):609-614 1982

Treatment of rats with carbon tetrachloride (CCl4) resulted in early reproducible losses of either one or two specific polypeptides (depending on the inducing agent with which the animals had been treated) in the mol wt range of the multiple forms of cytochrome P-450. The loss was correlated with a decrease in total cytochrome P-450 content in the microsome. The results of this study and those in the accompanying report indicate that CCl4 was metabolized by a specific form of cytochrome P-450 (52,000 daltons), which was rapidly destroyed in the process. The early loss of this peptide occurred simultaneously with the previously demonstrated production of highly reactive trichloromethyl radicals (•CCl3). This polypeptide, which was shown to disappear from liver microsomes following treatment of rats with CCl4, was demonstrated in the accompanying report to be the form of cytochrome P-450 specifically required for production of the highly reactive trichloromethyl radical in a reconstituted monooxygenase system. (Author abstract) (25 Refs)

43. SPECIFICITY OF A PHENOBARBITAL-INDUCED CYTOCHROME P-450 FOR METABOLISM OF CARBON TETRACHLORIDE TO THE TRICHLOROMETHYL RADICAL.

Noguchi T, Fong KL, Lai EK, Alexander SS, King MM, Olson L, Poyer JL, McCay PB
(c/o McCay) Biomembrane Res. Lab., Oklahoma Medical Res. Foundation, Oklahoma City, OK, 73104
Biochem Pharmacol; 31(5):615-624 1982

Evidence is presented which demonstrates that the first polypeptide to disappear in liver microsomes of phenobarbital-induced rats treated with CCl4 was the 52,000 dalton P-450 cytochrome. Data are also presented which show that this form of cytochrome P-450 was capable of generating the trichloromethyl radical from CCl4 in a reconstituted system containing the purified cytochrome, NADPH-cytochrome P-450 reductase, NADPH, CCl4, and the spin-trapping agent, phenyl-t-butyl nitrone. Other cytochrome P-450 fractions not containing the 52,000 dalton form did not produce this radical. The formation of this highly reactive radical may have resulted in localized damage to the cytochrome, causing the cytochrome either to be released from the microsomal membrane or to form large aggregates which did not migrate in the gel electrophoretic procedures employed. (Author abstract) (30 Refs)

44. PURIFICATION AND CHARACTERIZATION OF A SECOND FORM OF HEPATIC CYTOCHROME P-448 FROM RATS TREATED WITH A PURE POLYCHLORINATED BIPHENYL ISOMER.

Goldstein JA, Linko P, Luster MI, Sundheimer DW
Natl. Inst. Environmental Health Sciences, Research Triangle Park, NC, 27709
J Biol Chem; 257(5):2702-2707 1982

A new form of cytochrome P-448 has been isolated from livers of rats treated with 3,4,5,3',4',5'-hexachlorobiphenyl (HCB). The purified cytochrome (referred to as P-448HCB) had a specific content of 18.8 nmol of cytochrome/mg of protein and migrated as a single band on sodium dodecyl sulfate-polyacrylamide gels. The major forms of cytochrome P-450 induced by phenobarbital and 3-MC (referred to as P-450PB and P-450MC, respectively) were also purified for comparative studies. Although cytochrome P-448HCB has a mol wt similar to that of cytochrome P-450PB, these cytochromes differ in their spectral properties, chromatographic behavior, and peptide maps after limited proteolysis. Cytochrome P-448HCB clearly differs from cytochrome P-448MC in its mol wt, chromatographic behavior, and peptide maps. The oxidized spectra of cytochromes P-448MC and P-448HCB are also distinctly different. Oxidized cytochrome P-448HCB has absorbance maxima at 393 and 648 nm, indicating that this form is a high spin ferric hemoprotein. In contrast, cytochrome P-448MC has a Soret max at 418 nm, indicative of the low spin state. Antisera against cytochrome P-448HCB gave a single precipitin band against its antigen in Ouchterlony double diffusion plates but did not cross-react against cytochrome P-450PB. A faint cross-reaction against cytochrome P-448 was observed. This cross-reaction could be removed by immunoabsorption with partially purified cytochrome P-448MC indicating that the three hemoproteins have different immunological properties. (29 Refs)

45. DIFFERENTIAL INDUCTION OF RAT HEPATIC CYTOCHROME P-448 AND GLUTATHIONE S-TRANSFERASE B MESSENGER RNAS BY 3-METHYLCHOLANTHRENE.

Pickett CB, Telakowski-Hopkins CA, Donohue AM, Lu AY, Hales BF
Dept. Biochemical Regulation, Merck Sharp and Dohme Res. Lab., Rahway, NJ, 07065
Biochem Biophys Res Commun; 104(2):611-619 1981

The in vitro levels of functional rat liver cytochrome P-448 and glutathione S-transferase B messenger(m)RNAs in response to 3-methylcholanthrene (3-MC) were quantitated by polyadenylate-RNA translation and specific immunoprecipitation with rabbit anti-cytochrome P-448 IgG. Administration of 40 mg/kg 3-MC to rats resulted in an accumulation of functional mRNAs specific for cytochrome P-448 and glutathione S-transferase B; 3-MC administration lowered rat serum albumin mRNA. Fluorograms of sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed selective immunoprecipitation of two polypeptides of mol wt 55,000 and 54,000 daltons, respectively, from the in vitro translation system programmed with total liver polyadenylated-RNA from 3-MC-treated rats. No such polypeptides were precipitated from the translation system of untreated rats. Similar analysis revealed two primary translation products of glutathione S-transferase B, of 29,000 and 27,000 dalton mol wt, which were markedly more concentrated in the translation mixtures from 3-MC-treated rats. The elevation of immunoreactive cytochrome P-448 and glutathione S-transferase B after 3-MC treatment can be totally accounted for by increases in the level of mRNA coding the proteins. (18 Refs)

46. ALTERATION OF EPIDERMAL GROWTH FACTOR-DEPENDENT PHOSPHORYLATION DURING RAT LIVER REGENERATION.

Rubin RA, O'Keefe EJ, Earp HS
Cancer Cell Biology Pogram, Cancer Res. Center, Univ. North Carolina, Chapel Hill, NC, 27514
Proc Natl Acad Sci USA; 79(3):776-780 1982

Epidermal growth factor (EGF) stimulates membrane protein phosphorylation in a human cell line, A-431. The known hepatic mitogenic action of EGF and the reduction in EGF receptor number that occurs during liver regeneration led us to study whether EGF-dependent protein kinase activity was present in rat liver and whether its activity was altered after partial hepatectomy. Liver membranes preincubated with or without EGF, were phosphorylated (0 C, 15 sec) and subjected to NaDodSO4/polyacrylamide gel electrophoresis and autoradiography. In microsomal fractions, EGF at 5-2,000 ng/ml produced a dose-related stimulation of 32P incorporation into a single 170,000-dalton protein (p170). In plasma membranes, a similar EGF-dependent phosphorylation was present and was substantially enriched relative to the microsomal fraction. Acid hydrolysis of labeled microsomal fraction followed by phosphoamino acid determination revealed that EGF stimulated 32P incorporation into phosphotyrosine residues. The EGF-dependent phosphorylation of p170 was compared in microsomal fractions isolated from rats 36 hr after partial hepatectomy or sham operation. In the absence of EGF, in vitro labeling of p170 was similar. EGF stimulated the labeling of p170 in both groups, but the response was clearly diminished after partial hepatectomy. In the presence of EGF, the labeling of p170 in microsomal fraction from regenerating livers was only 47 -6% of that observed in membranes from sham-operated rats (p less than 0.005). Reduction of EGF-dependent phosphorylation during liver regeneration paralleled the loss of binding of 125I-labeled EGF. An increase in the EGF-dependent phosphorylation of a 130,000-dalton protein was also observed after partial hepatectomy. The increase in the amount of this phosphoprotein was roughly equal to the loss of EGF-stimulated p170 phosphorylation. Several additional proteins showed increased phosphorylation in membranes from partially hepatectomized rats. These findings indicate that alterations in membrane tyrosine residue phosphorylation occur during regulated growth in vivo. (Author abstract) (28 Refs)

47. ALTERATION OF PROTEIN SYNTHESIS AND INDUCTION OF SPECIFIC PROTEIN PHOSPHORYLATION BY HYPERTHERMIA.

Rubin I, Getz G, Swift H
Dept. Pathology, Univ. Chicago, Chicago, IL, 60637
Cancer Res; 42(4):1395-1398 1982

Confluent cultures of the mouse cell line clone 1D were subjected to 1-hr hyperthermic treatments. Temperatures were increased from the control level of 37 C to values ranging from 38 to 45 C. Protein synthesis patterns were determined in fluorograms of sodium dodecyl sulfate-polyacrylamide gels labeled with [3H]leucine. Although incorporation into most proteins as either repressed or decreased by heat treatment, several proteins showed an increased label or were apparently induced de novo. Among the induced proteins was a prominent band, probably a doublet, with an estimated mol wt of 70,000 to 69,000. Crude cell lysates made from 37 C, 41 C, and 45 C-treated cells were tested for kinase activity at 30 C by a 10-min incubation with adenosine [gamma-32P]triphosphate. Several specific proteins exhibited increased phosphorylation, while phosphorylation of other proteins decreased. The most significant increase in phosphorylation was shown by a protein with mol wt of about 37,000. We suggest that heat treatment induces or activates one or more specific phosphokinase(s) with the ability to phosphorylate proteins with approx mol wt o f 37,000, 36,000, 23,000 and 16,000. (Author abstract) (24 Refs)

48. THERMOTOLERANCE AND HEAT SHOCK PROTEINS INDUCED BY HYPERTHERMIA IN RAT LIVER CELLS.

Landry J, Chretien P, Bernier D, Nicole LM, Marceau N, Tanguay RM
Cell Biophysics, Molecular Oncology Res. Center, Hotel-Dieu de Quebec, 11, Cote du Palais, Quebec G1R 2J6, Canada
Int J Radiat Oncol Biol Phys; 8(1):59-62 1982

The correlation between the development of thermotolerance and increased protein synthesis was studied in propagable hepatic epithelial cells isolated from minced liver from a newborn Fisher 344 rat (NN/La). NN/La cells were exposed to a conditioning treatment at 43 C for 30 min and returned to 37 C. The conditioning treatment induced a dramatic increase in thermotolerance in NN/La cells. The effect developed fully within 4-8 hr at 37 C, remained at a maximal level for greater than 1 day, and gradually disappeared during day 3. At full development, the resistant cells tolerated hyperthermia 50x more efficiently than the normal unconditioned cells. The progressive increase in thermotolerance was accompanied by an elevated synthesis of particular proteins. During this period, the electrophoretic pattern revealed five new labelled proteins with mol wt of 107, 89, 70, 68, and 27 kilodaltons (KD). The duration of the increased synthesis induction was 1-2 hr for the 27 KD protein and up to 8 hr for the 89 KD protein. A heat-induced increase in protein synthesis of a small set of proteins also was observed in diploid rat epithelial cell strain RL-PR-C derived from livers of newborn rats, in MH-7777 cells derived from the Morris hepatoma 7777, and in hepatocytes from Fisher 344 rats. Accumulation of the proteins in the cells may play a role in the process leading to a thermotolerant state. (21 Refs)

49. INDUCED THERMAL TOLERANCE AND HEAT SHOCK PROTEIN SYNTHESIS IN CHINESE HAMSTER OVARY CELLS.

Li GC, Petersen NS, Mitchell HK
Dept. Radiation Oncology, Univ. California, San Francisco, CA, 94143
Int J Radiat Oncol Biol Phys; 8(1):63-67 1982

Kinetics of induction of thermal tolerance in Chinese hamster HA-1 cells and effects of heat treatments on the recovery of protein synthesis were studied. Plateau phase HA-1 cells growing in monolayers were exposed to 45 C for 20 min. Some of the cells were then challenged immediately by a second treatment at 45 C (30 min) while others were incubated at 37 C for 1-8 hr before the second heat challenge. A comparison of the proteins synthesized after heat shock (HSP) with those synthesized by non-heated cells showed that the levels of synthesis of certain proteins were greatly enhanced following the 45 C treatment. By 8 hr, it was qualitatively apparent that three proteins with mol wt of 59,000, 70,000, and 87,000 dalton were synthesized in greater amounts than in untreated cells. By 8 hr the rates of synthesis of 59 ,000, 70,000, 87,000 dalton proteins and actin were 140%, 160%, 115%, and 53% of the rates in non-heated controls. The rate of development of thermal tolerance correlated well with the kinetics of HSP synthesis. (21 Refs)

DIETARY AND NUTRITIONAL EFFECTS

50. ALTERED AMINO ACID KINETICS IN RATS WITH PROGRESSIVE TUMOR GROWTH.

Kawamura I, Moldawer LL, Kennan RA, Batist G, Bothe A, Bistrian BR, Blackburn GL
(c/o Blackburn), Cancer Res. Inst., 194 Pilgrim Road, Boston, MA, 02215
Cancer Res; 42(3):824-829 1982

The present study was designed to determine whether alterations in host metabolism associated with progressive tumor growth were a result of the anorexia frequently observed with cancer or could be attributed to other direct tumor effects. Rates of tyrosine flux, oxidation, and incorporation into protein, as well as fractional protein-synthetic rates in nonsecretory liver, muscle, and tumor, were determined in overnight-fasted rats, 5 to 6 (Stage I), 10 to 11 (Stage II), and 15 to 16 (Stage III) days following sc implantation of RNC-254 fibrosarcoma. Tumor-bearing rats were allowed to consume a purified diet containing 20% protein ad libitum, and results were compared to non-tumor-bearing rats pair fed quantities of food equivalent to tumor-bearing animals or allowed to consume the diet ad libitum. Results demonstrate that during later stages of tumor growth (Stage III) cal intake and nontumor body wt gain were reduced in tumor-bearing rats (p less than 0.05). Fifteen and 16 days following implantation, there were significant changes in amino acid kinetics that were not observed after earlier periods of tumor growth and that could not be explained by any reduction in dietary intake. Rates of tyrosine appearance in the plasma and subsequent incorporation into whole-body protein were increased 33 and 34%, respectively (p less than 0.05), when compared to non-tumor-bearing rats fed equivalent quantities of food. Whole-body tyrosine oxidation rates were unchanged. Skeletal protein synthesis, as reflected by gastrocnemius or rectus abdominus muscle, was reduced from 10.5 and 10.1%/day to 7.4 and 6.0%/day, respectively (p less than 0.05), in tumor-bearing compared to pair-fed animals. The findings suggest that significant alterations in protein metabolism occur in advanced stages of experimental neoplastic disease which cannot be explained by reductions in dietary intake and are aimed at providing adequate quantities of endogenous amino acids for net tumor growth. (Author abstract) (32 Refs)

51. PARENTERAL NUTRITION AND HUMAN GASTROINTESTINAL TUMOR PROTEIN METABOLISM.

Stein TP, Buzby GP, Leskiw MJ, Mullen JL
Surgical Res. Lab., Graduate Hosp., Univ. Pennsylvania, 19th and Lombard Sts., Philadelphia, PA, 19146
Cancer; 49(7):1476-1480 1982

We determined the tissue free amino acid distribution in human gastrointestinal tumors and the adjacent normal tissue in two groups of patients. The patients were maintained on dietary control for 7-10 days prior to the surgical removal of the tumor. Patients in Group I ate the house diet ad lib. Their nutritional intake was about half their normal requirement. The second group received parenteral nutrition. Both groups were equally malnourished at the beginning of the period of nutritional control. Group I were 88.2 -7.8% of ideal body weight and Group II, 90.6 -13.8% of ideal body weight. The net calorie and nitrogen intake was about double that of Group I. Tissue-free amino acid analyses were done by gas chromatography. Results: There was a statistically significant increase in the essential amino acids in the tumor tissue relative to the normal tissue in Group II, in contrast to the orally fed Group I where no discernible differences were found. (Author abstract) (15 Refs)

52. POSTNATAL DEVELOPMENT OF THE VENTRAL PROSTATE GLAND IN NORMAL VERSUS PROTEIN-CALORIE MALNOURISHED RATS.

Weaker FJ, Herbert DC
Dept. Anatomy, Univ. Texas Health Science Center at Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78284
Prostate; 2(3):249-260 1981

Sprague-Dawley rats were subjected to a low-protein diet of 8% protein, 78% starch, 10% vegetable oil, and 4% salt mixture, and every 7 days from day 27 to day 55 sections of the ventral prostate gland (VPG) were examined by light and electron microscopy. Controls were fed an isocaloric standard diet with 27% protein. Height of the epithelium and fine structural appearance varied widely, but specific morphological features were apparent which distinguished the VPGs of rats fed the standard diet from those fed the low-protein diet. Ultrastructural differences between the two groups first became apparent on day 34. From this time onward the amount of rough endoplasmic reticulum, size of the Golgi complex, number of secretory granules, and diameter of nuclei were all reduced in secretory cells of the VPGs of malnourished rats compared to controls. The apparent size and number of condensing flocculent and myelin-containing vacuoles increased throughout the course of the study in the epithelial cells of malnourished rats. Morphological alterations were not as severe as reported after castration, but by 55 days of age prostate glands of protein-calorie malnourished rats resembled those of prepubertal animals. (19 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

53. METABOLISM OF ACETYL DERIVATIVES OF POLYAMINES IN CULTURED POLYAMINE-DEFICIENT RAT HEPATOMA CELLS.

Mamont PS, Seiler N, Siat M, Joder-Ohlenbusch AM, Knodgen B
Centre de Recherche Merrel International, 16, rue d'Ankara, 67084 Strasbourg Cedex, France
Med Biol; 59(5/6):347-353 1981

Rat hepatoma tissue culture (HTC) cells depleted of their putrescine and spermidine content by the use of DL-alpha-difluoromethylornithine (DFMeOrn) do not respond to exogenously added spermidine or spermine by restoration of cellular putrescine and spermidine, respectively. Comparative studies of exogenously added spermidine, N1-acetylspermidine, monoacetylputrescine, or N1-N12-diacetylspermine to HTC cells cultured for 72 hr in the presence of 5 milliM DFMeOrn confirm the presence of an in situ fully active polyamine oxidase, which preferentially cleaves N1-acetyl derivatives of polyamines. Acetyl polyamines were stable in dialyzed horse serum, but spontaneous transformations occurred in newborn calf serum, indicating that care must be taken in interpreting short-term studies using calf serum. The preferential cleavage in situ by polyamine oxidase of N1-acetyl derivatives of polyamines compared to non-acetylated polyamines was demonstrated; it appears that acetylation may be the first step in degradation of polyamines. (34 Refs)

54. EFFECT OF POLYAMINES ON ENZYMES INVOLVED IN DNA REPAIR.

Kleppe K, Osland A, Fosse V, Male R, Lossius I, Helland D, Lillehaug JR, Raae AJ, Kleppe RK, Nes IF
Dept. Biochemistry, Univ. Bergen, Bergen, Norway
Med Biol; 59(5/6):374-380 1981

The effects of polyamines on UV incision nuclease, DNA polymerase I, DNA ligase and polynucleotide kinase, apurinic- or apyrimidinic (AP)- endonucleases, and enzymes involved in folding and unfolding of chromosomes are reviewed. Polyamine-induced cleavage of AP-sites is also discussed. Spermine and spermidine have been found to be more efficient in causing hydrolysis of DNA than cadaverine or putrescine. (29 Refs)

55. ROLE OF POLYAMINES IN HELA CELL PROLIFERATION.

Herbst EJ, Elliot QD
Dept. Biochemistry, Univ. New Hampshire, Durham, NH
Med Biol; 59(5/6):410-416 1981

Nuclei isolated from HeLa cells by the method of Krokan were severely depleted of polyamines by administration of alpha-difluoromethylornithine (DFMO), the irreversible inhibitor of ornithine decarboxylase. Simultaneously, cells were synchronized for S phase DNA synthesis by a double thymidine block procedure. The inhibitor of S phase DNA synthesis in nuclei isolated from cells synchronized in media containing

DFMO appears to be caused by depletion of polyamines in these cells. Replenishment of intracellular polyamines, especially spermidine, by administration of exogenous putrescine, spermidine, or spermine overcomes the DNA replication deficiency. The effect of polyamines is evidently indirect; a role in RNA polymerase I activity is possible. (29 Refs)

56. POLYAMINES MODIFY PARAQUAT-INDUCED CHANGES IN PULMONARY SUPEROXIDE DISMUTASE AND LIPID PEROXIDATION.

Khanna YP, Sushil K, Raj TH, Venkitasubramanian TA
(c/o Raj) Dept. Biochemistry, Vallabhbhai Patel Chest Inst., Univ. Delhi, Delhi 110 007, India
Res Commun Chem Pathol Pharmacol; 35(2):337-340 1982

Lungs of rats intoxicated by paraquat either due to parenteral administration or incubation of tissue slices with the toxin appear to be responsive to the action of polyamines. Putrescine spermidine and spermine were able to significantly counter paraquat-induced augmentation of lipid peroxidation and superoxide dismutase (SOD) activity in the lungs of rats. Non-physiological polyamines like cadaverine and agmatine were also found to diminish peroxidation of lipids as well as SOD activity stimulated by paraquat. The protective action of polyamines could be due to membrane stabilization and prevention from oxidant damage. (Author abstract) (9 Refs)

OTHER RELATED STUDIES

57. ASSAYS OF CELL SURFACE PROTEIN KINASE: IMPORTANCE OF SELECTING CYTOPHILIC SUBSTRATES.

Kubler D, Pyerin W, Kinzel V
Abt. fur Molekulaire Pathologie, Institut fur Experimentelle Pathologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Fedl 280, D-6900 Heidelberg 1, W. Germany
Eur J Cell Biol; 26(2):306-309 1982

The ability of phosvitin and histone to serve as substrates for possible protein kinase(s) at the cell surface was examined with regard to their suitability as indicators of such activity. While phosvitin did not interfere with the membrane barrier of HeLa cells, 3T3 and SV 3T3 cells, histone caused severe damage as indicated by both uptake of viability stains Trypan Blue and diamidino-phenylindol and by release of intracellular compounds such as lactate dehydrogenase, cAMP-dependent protein kinase(s), and metabolically prelabeled proteins. Where intactness of the cell membrane is prerequisite for verification of ecto-protein kinase, histone cannot be used as substrate. In contrast, we found that phosvitin is suitable for assays of cell surface located protein kinase activity. (Author abstract) (22 Refs)

58. HIGH UPTAKE OF 14C-LABELED D-AMINO ACIDS BY VARIOUS TUMORS.

Tamemasa O, Goto R, Takeda A, Maruo K
Div. Radiobiochemistry and Radiopharmacology, Shizuoka Coll. Pharmacy, Oshika 2-2-1, Shizuoka 422, Japan
Gan; 73(1):147-152 1982

As an extension of the previous finding that radioactivity of 14C-labeled D-amino acids after injection is localized preferentially in the tumor and the pancreas of tumor-bearing animals as compared with the corresponding L-amino acids tested, the results of similar uptake experiments using other tumors are reported here. The present studies show high radioactivity uptake by human colon cancer, human thyroid cancer, and human leiomyosarcoma transplanted into nude mice, and by solid leukemia L1210 and solid sarcoma 180, but not by Morris hepatoma 7316A or 3'-methyl-4-(dimethylamino)azobenzene-induced rat hepatoma. The results suggest the potential utility of 14C-labeled D-amino acids for the detection of some can cers. (Author abstract) (14 Refs)

59. CORRELATION BETWEEN D REGION STRUCTURE AND ANTIGEN-BINDING SPECIFICITY: EVIDENCES FROM THE COMPARISON OF CLOSELY RELATED IMMUNOGLOBULIN VH SEQUENCES.

Auffray C, Sikorav JL, Ollo R, Rougeon F

Unite de Genetique et Biochimie de Developpement, E.R.A. CNRS 851, Institut Pasteur, 75724 Paris Cedex 15, France

Ann Immunol (Paris); 132(1):77-88 1981

Complementary DNA (cDNA) clones containing VH sequences of three myeloma proteins, of which two (UPC10 and ABE48) bind 2,6-levan, and one (MOPC173) has an unknown specificity, were constructed and sequenced. cDNA clones were compared to four sequences of anti-galactan myeloma proteins belonging to NIH BALB/c plasmacytoma. Starting with partially purified alpha heavy chain messenger RNA (mRNA) from ABE48 myeloma and gamma2a heavy chain mRNA from MOPC173 myeloma, collections of recombinant plasmids were constructed. Plasmids palpha48-16 and pG2a-173-27, which carry the most important part of the corresponding VH sequences were isolated using 650-base pair PstI fragment of pG2a-10-21 containing the VH sequence. Nucleotide sequences are presented. As a result of the cloning procedure, the sequence corresponding to the 5' end of the mRNA is not present in pG2a-173-27, which begins at the codon 5 of the variable region of the heavy chian (VH region), and in palpha48-16, which begins at codon 32 of the VH region. The amino-acid sequences presented were derived from the nucleotide sequences. The MOPC173 sequence agrees with a previously reported except for the exchange Asn/Asp at codons 52-54 and 73-74. Results confirm the strong correlation existing between D-region structure and antigen-binding specificity. (29 Refs)

60. TRANSPORT OF AN IMMUNOGLOBULIN LIGHT CHAIN FRAGMENT ACROSS THE ENDOPLASMIC RETICULUM DOES NOT REQUIRE AN AMINO TERMINAL VARIABLE REGION: IMPLICATIONS FOR THE SIGNAL HYPOTHESIS.

Fetherston J, Boime I

Dept. Pharmacology, Washington Univ. Medical Sch., 660 S. Euclid Ave., St. Louis, MO, 63110

Biochem Biophys Res Commun; 104(4):1630-1637 1982

The co-translational processing of the kappa light chain constant region fragment was studied in a Krebs ascites cell-free system. The fragment (which is devoid of the entire variable region) was initially synthesized as a precursor which contains a signal (pre) peptide. In the presence of microsomal membranes, the pre-peptide was removed and the cleaved fragment was transported into the vesicles. NH2-terminal sequences normally adjacent to the signal peptide of a kappa light chain were not necessary for translocation. (23 Refs)

61. EFFECT OF TOXIN-ANTIBODY CONJUGATES ON TUMOR CELLS.

Uhr JW
Dept. Microbiology, Southwestern Medical Sch., Univ. Texas Health Science Center, Dallas, TX, 75235
Cell Immunol; 66(1):24-28 1982

An attempt was made to use a specific antibody to deliver a toxin (ricin, a highly toxic plant lectin) to tumor cells. Conjugates (anti-muA) of rabbit anti-mu and A chains of ricin were prepared. Murine B lymphocytes bearing IgM were exposed to anti-muA. A binding assay showed that the conjugate retained specificity for IgM. Toxicity was assayed by stimulating BALB/c mouse spleen cells with lipopolysaccharide and then exposing the cells to anti-muA. Anti-muA at concentrations of 5-10 ug/ml inhibited protein synthesis in the cells by 80%. Protein synthesis was not inhibited by treatment with conjugates of normal rabbit immunoglobulin and A chain or with unconjugated antibody. Since ricin functions by inhibition of protein synthesis, results indicate that anti-muA retained antigen-specific toxicity. Spleen cells from mice bearing BCL1 tumors were exposed in vitro to conjugates of anti-idiotype antibodies and A chains (anti-iA). Anti-iA inhibited protein synthesis in the spleen cells by 70%. Since spleens from the tumor-bearing mice consisted of approx 70% BCL1 cells, results indicate that anti-iA is capable of specific destruction of a particular population of tumor cells. A strategy of nonimmunologic reduction of pathologic cell population followed by elimination of surviving cells with anti-idiotypic-ricin conjugates is suggested for treatment of leukemia. (4 Refs)

62. REPLICATION AND PHENOTYPIC EXPRESSION OF CONTROL AND SCLERODERMA HUMAN FIBROBLASTS: RESPONSES TO GROWTH FACTORS.

LeRoy EC, Mercurio S, Sherer GK
Div. Rheumatology and Immunology, Dept. Medicine, Medical Univ. South Carolina, 171 Ashley Ave., Charleston, SC, 29425
Proc Natl Acad Sci USA; 79(4,part1):1286-1290 1982

To explore the mechanism of increased collagen synthesis by scleroderma skin fibroblasts in vitro, control and scleroderma fibroblasts were compared in confluent monolayer cultures growth-arrested by serum deprivation; responses to optimal mitogenic doses of platelet-derived growth factor, fibroblast growth factor, epidermal growth factor and nerve growth factor were compared. Platelet-derived growth factor had a selective mitogenic effect on control skin fibroblasts not observed with scleroderma skin fibroblasts. None of the factors studied had a selective effect on collagen synthesis independent of cell replication; scleroderma and control fibroblasts responded similarly. Therefore, the growth factors studied may not be involved in generating the activated scleroderma fibroblast directly; platelet-derived growth factor may play an indirect role in fibroblast replication in human fibrotic disorders. (Author abstract) (30 Refs)

63. CHANGES IN PLASMA AMINO ACID CONCENTRATIONS AFTER ABDOMINAL OPERATIONS.

Iriyama K, Teranishi T, Mori H, Takenaka T, Mori T
Second Dept. Surgery, Mie Univ. Sch. Medicine, Tsu, Japan
Mie Med J; 31(1):207-211 1981

Postoperative changes in plasma levels of amino acids were studied in four patients undergoing subtotal gastrectomy and lymph node dissection for gastric cancer, and one patient undergoing gastrectomy and cholecystectomy for gastroduodenal ulcer and gall stones. On day 3 after surgery significant decreases in threonine, glutamic acid, serine, proline, valine, leucine, isoleucine, histidine, lysine, and arginine were detected. Changes in amino acid levels indicated catabolism and were not similar to those produced by simple starvation. (8 Refs)

64. CHANGES IN GENE EXPRESSION ACCOMPANYING CHEMICALLY-INDUCED MALIGNANT TRANSFORMATION OF HUMAN FIBROBLASTS.

Leavitt J, Goldman D, Merril C, Kakunaga T
Linus Pauling Inst. Science and Medicine, Palo Alto, CA, 94306
Carcinogenesis; 3(1):61-70 1982

The modulation of gene expression accompanying neoplastic transformation has been assessed by computerized microdensitometry of autoradiographic patterns of [35S]methionine labeled polypeptides separated by two-dimensional polyacrylamide gel electrophoresis. Nearly 1,000 polypeptide species of parent diploid human fibroblasts (KD strain) and clonally-derived malignant fibroblast (HUT-14 strain) were compared. HUT-14 fibroblasts express a mutation in one of the two functional beta-actin genes and possess properties that distinguish them as neoplastic cells. Of the 700 more abundant polypeptides measured, 13 were lost and 14 were gained following this neoplastic transformation. It is estimated that less than or equal to 2% of the genes expressing abundant polypeptides were either activated or shut off, but at least 32% were modulated quantitatively as a consequence of this neoplastic transformation. Classes of 'highly variable' and 'marginally variable' polypeptides were assigned. Among the 'highly variable' polypeptides, two related species barely detectable in KD parental cells were synthesized at a 25-31-fold higher rate in the transformed cells, and the cell-associated and extracellular matrix forms of fibronectin were each diminished by greater than 90%. Principles emerging from this study may form a basis for interpretation of the role of individual genes in the expression of neoplastic characteristics in HUT-14 cells. (Author abstract) (29 Refs)

65. ALTERATIONS OF POLYPEPTIDE COMPOSITION OF MATURE GRANULOCYTES OBTAINED FROM PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA.

Yokota J, Asano S, Teshima S, Morishita K, Iwamoto A, Yoshikura H, Miwa S
Dept. Genetics, Inst. Medical Science, Univ. Tokyo, 6-1, 4 Shiroganedai, Minato-ku, Tokyo 108, Japan
Blood; 59(2):443-446 1982

In order to compare the polypeptide composition of the CML mature granulocytes with that of the normal whole cell lysates, mature granulocytes from four healthy volunteers and six CML patients were analyzed by two-dimensional gel electrophoresis. In the normal subjects, 60 major spots were commonly and reproducibly identified. In the CML, there were constant alterations in some of these major spots. Four spots were totally absent in all the CML samples, and another four spots were absent in five of the six samples. In addition, one spot was larger in CML than in normal cells, and another spot, which was only faintly visible or not detectable in the normal samples, was massively present in all the CML samples. Our data suggest specific changes in the polypeptide compositions of CML granulocytes. This method could be clinically applied for the analysis of granulocytic disorders. (Author abstract) (11 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Alexander SS 43
Alitalo K 12
Andersen MR 9
Asano S 65
Atkin CL 9
Auffray C 59
Babusikova O 30
Batist G 50
Bender BL 22
Bergel'son LD 7
Berger G 13
Bernier D 48
Bistrian BR 50
Bjork P 20
Blackburn GL 50
Blinov MN 37
Boime I 60
Bothe A 50
Bravo R 4
Bremond A 13
Buzby GP 51
Carlin B 22
Carlstrom K 20
Celis JE 4
Chevalier M 13
Chorvath B 30
Chretien P 48
Chung AE 22
Colston K 23
Colston MJ 23
Creissen D 2
Denk H 6
Dermer GB 39
Diatlovitskaia 7
Donohue AM 45
Duraj J 30
Earp HS 46
Eggena P 10
Elliot QD 55
Eyre HJ 9
Feldman D 23
Fernandez-Pol J 32
Ferns SA 14
Feroldi J 13
Fetherston J 60
Fey SJ 4
Fieldsteel AH 23
Fong KL 42
Fong KL 43
Forchhammer J 31
Forsgren B 20
Fosse V 54
Franke WW 6
Frappart L 13
Freshney RI 5
Getz G 47
Gil L 40
Golde DW 10
Goldman D 64
Goldstein JA 44
Goodwin WJ 25
Goto R 58
Graham DI 5
Griffin AC 25
Grimaud JA 13
Grofova M 31
Guroff G 1
Gustafsson JA 20
Hales BF 45
Halila H 12
Hashimoto S 1
Helland D 54
Herbert DC 52
Herbst EJ 55
Hogberg B 20
Holland JS 33
Humphries DE 21
Iakimenko EF 7
Iriyama K 63
Ito H 26
Ivarie RD 38
Iwamoto A 65
Jaffe R 22
Jerkovic M 19
Jesse RH 25
Joder-Ohlenbusc 53
Johnson HM 8
Kacena A 10
Kakunaga T 64
Kawamura I 50
Kennan RA 50
Khanna YP 56
King MM 43
Kinzel V 57
Kleppe K 54
Kleppe RK 54
Knodgen B 53
Knox P 16
Kobata A 27
Kodama T 26
Kondo Y 18
Krepler R 6
Kubler D 57
Kuprina NI 7
Lai EK 42
Lai EK 43
Landry J 48
Larsen PM 4
LeRoy EC 62
Leavitt J 64
Leskiw MJ 51
Lesley J 34
Li GC 49
Lillehaug JR 54
Linko P 44
Litvak S 40
Lizonova A 31
Lloyd WS 21
Lossius I 54
Lu AY 45
Lusis AJ 10
Luster MI 44
Maeda S 27
Mage M 11
Male R 54
Mamont PS 53
Marceau N 48
Martial JA 38
Maruo K 58
Maruoka M 26
Mathieson B 11
Matsumoto A 27
Matsumura T 26
McCay PB 42
McCay PB 43
McNamee HB 5
Mercurio S 62
Merril C 64
Mitchell HK 49
Miwa S 65
Miyauchi T 26
Moldawer LL 50
Mori H 63
Mori T 63
Morishita K 65
Morozova EO 37
Morris JA 38
Mort JS 17
Mullen JL 51
Naito H 26
Nes IF 54
Nicole LM 48
Nimberg RB 21
Noguchi T 42
Noguchi T 43
Nozumi K 26
O'Keefe EJ 46
Ohnuma T 33
Ohsawa N 18
Ohtsuki K 8
Ollo R 59
Olson L 42
Olson L 43
Ong DE 24
Ong DE 25
Oppenheim JJ 11
Osland A 54
Petersen NS 49
Pickett CB 45
Poole AR 17
Popovic M 31
Pousette A 20
Poyer JL 43
Pyerin W 57
Raae AJ 54
Raj TH 56
Recklies AD 17
Rees KR 41
Rieber M 36
Rieber MS 36
Robinson A 3
Rochet Y 13
Rougeon F 59
Rubin I 47
Rubin RA 46
Rudland PS 14
Salazar I 40
Salisbury JG 29

Sato R 26
Scher BM 3
Scher W 3
Schmid E 6
Schmid K 21
Schweizer J 15
Seiler N 53
Serafini-Fracas 16
Shall S 2
Sherbet GV 35
Sherer GK 62
Shimazaki J 26
Shiraishi N 27
Siat M 53
Sikorav JL 59
Sinitsyna EV 7
Siraganian RP 11
Slankard-Chahin 33
Small JV 4
Spolter L 10
Stadler BM 11
Stefansson K 19
Stein TP 51
Stockbauer P 30
Sugiyama T 27
Sun AS 33
Sundheimer DW 44
Sushil K 56
Swift H 47
Takeda A 58
Takeichi M 28
Takenaka T 63
Tamemasa O 58
Tanguay RM 48
Tarrago-Litvak 40
Telakowski-Hopk 45
Temple T 22
Teranishi T 63
Teshima S 65
Timofeeva NG 7
Torres BA 8
Trowbridge IS 34
Uhr JW 61
Vaheri A 12
Vaughan PF 5
Venkitasubraman 56
Vesterinen E 12
Wakisaka M 26
Warburton MJ 14
Waxman S 3
Weaker FJ 52
Weirich E 6
Weisbart RH 10
Wells H 21
Wells P 16
West GM 5
White JS 41
Wildridge M 35
Winter H 15
Wollmann R 19
Yokota J 65
Yoshida C 28
Yoshikura H 65
Yoshima H 27
Zenisek SC 32

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INDEXES

Tumor index
Agent/Modality index
Protocol identification number index

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MOLECULAR BIOLOGY

Proteins, Polypeptides, Amino Acids

SERIES CB27 No. 82/08

AUGUST, 1982

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In this issue:

- **NUCLEOPROTEINS** **1-5**
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** **6-18**
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** **19-30**
- **BINDING/ RECEPTOR PROTEINS** **31-35**
- **MEMBRANE/ SURFACE PROTEINS** **36-49**
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** **50-55**
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** **56-60**
- **DIETARY AND NUTRITIONAL EFFECTS** **61-62**
- **POLYAMINES - SYNTHESIS AND METABOLISM** **63**
- **OTHER RELATED STUDIES** **64-72**

NUCLEOPROTEINS

1. READILY EXTRACTABLE NONHISTONE PROTEINS IN NORMAL CHROMATIN AND IN CHROMATIN OF THE PATIENTS WITH DIFFERENT VARIANTS OF LEUKEMIA.

Poddubnaia EV, Iavorkovskii LI, Blumberga IA, Dron' LV
Medical Inst., Riga, USSR
Eksp Onkol; 4(1):32-35 1982

The results of comparative analysis of nonhistone proteins extracted with 0.35 M NaCl (0.35 NHP) from granulocytes of

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healthy donors and from peripheral blood leukocytes of acute leukemia (AL) patients are reported. The total content of 0.35 NHP in the granulocytes was significantly lower than that in the leukocytes of the patients with lymphoblastic and myeloblastic variants of AL. Electrophoresis in polyacrylamide gel showed that 0.35 NHP from normal granulocytes consisted of 18-20 fractions, compared with 22-27 fractions in AL. The patients with myeloblastic AL showed increase of the fractions with intermediate and low electrophoretic motility, while the patients with lymphoblastic variants of AL showed increase of the fast fractions. (26 Refs) (In RUS)

2. MICROSOMAL CAMP-INDEPENDENT HISTONE H1 KINASE ACTIVITY IN PLASMACYTOMA, MORRIS HEPATOMA AND NORMAL LIVER.

Schmitt M, Quirin-Stricker C, Kempf J

U-184 INSERM de Biologie Moleculaire et de Genie Genetique, Institut de Chimie Biologique, Faculte de Medecine, 11, rue Humann, 67085 Strasbourg Cedex, France

Biochimie; 64(1):13-20 1982

A protein kinase activity with high specificity for histone H1 was isolated from mouse plasmacytoma, Morris hepatoma and normal mouse liver and compared by ion exchange chromatography after DEAE-cellulose, hydroxylapatite and Sephadex G-200 chromatography. This cAMP-independent histone H1 kinase is not affected by the heat-stable cAMP-dependent protein kinase inhibitor. It has the following particular properties: it prefers GTP to ATP as substrate and was found to be present with a great activity only in neoplastic tissues. No phosphatase activity was detected in the partially purified histone H1 kinase fraction from normal and neoplastic cells. These results suggest either an increased amount of histone H1 kinase and/or of its activator in neoplastic cells, or the presence of a strong inhibitor in normal cells. This histone H1 kinase appears to be analogous to the chromatin bound kinase which phosphorylates histone H1 at the NH_2 and COOH terminal regions. We might suggest an implication of this kinase in the regulation of cell division. (Author abstract) (12 Refs)

3. ELECTROPHORETIC AND IMMUNOLOGIC CHARACTERISTICS OF NON-HISTONE PROTEINS OF HUMAN SKIN TUMORS.

Landi FL, Borrello S, Serri F

Istituto di Clinica Dermatologica, Universita Cattolica S. Cuore, Roma, Italy

Boll Soc Ital Biol Sper; 57(22):2298-2302 1981

Nine human skin tumors, surgically removed and less than 5 g in size were diagnosed clinically and histologically as melanotic and amelanotic melanomas or squamous epitheliomas. Methods used in isolation of cellular nuclei and nuclear proteins from the tumors were described. Two different methods were used for extraction of the nuclear proteins (single-step extraction and sequential-step extraction). Electrophoresis was performed according to a modified Laemly method. Immunostimulation was performed in adult New Zealand rabbits by injecting 2 mg of nuclear proteins of spinocellular epithelioma 6x at intervals of 2 wk after which blood was removed, separated, and the serum partially purified and concentrated 4x. (6 Refs) (In ITA)

4. ELECTROPHORETIC AND IMMUNOLOGIC CHARACTERISTICS OF NON-HISTONE PROTEINS IN HUMAN SKIN TUMORS. II. RESULTS.

Landi FL, Carnevali P, Bracaglia R, Borrello S, Serri F

Istituto di Clinica Dermatologica, Universita Cattolica del S. Cuore, Roma, Italy

Boll Soc Ital Biol Sper; 57(22):2303-2306 1981

Nine patients with skin tumors had melanoma (3), spinocellular epithelioma (SE: 4), intermediate epithelioma (1), or fibrous histiocytoma (1). Electrophoretic (EP) and immunologic (IM) studies were performed on non-histone nuclear proteins (NHNP) of the tumors according to methods published previously. EP profiles of the NHNP of the different tumors showed some common and some different bands. These EP bands varied quantitatively between different patients with the same type of skin tumor (SE) and between patients with different types of skin tumors. Immunodiffusion of the NHNP of the different skin tumors showed for all of them the same two analogous and symmetric precipitation bands. Antibodies induced by denatured nuclear proteins did not react with native nuclear proteins of the SE or with histones from liver nuclei of Wistar rats. Contrary to expectations, no significant differences were observed between the NHNP of different skin tumors. The NHNP responsible for cellular differentiation are very limited. (3 Refs) (In ITA)

5. NONHISTONE PROTEINS OF RAT LIVER TRANSFORMED BY NITROSODIETHYLAMINE.

Seits IF, Glebov AV

N. N. Petrov Res. Inst. Oncology, USSR Ministry Health, Leningrad, USSR

Vopr Onkol; 28(1):42-46 1982

Qualitative and quantitative content of nonhistone chromosomal proteins (NHP) from normal and nitrosodiethylamine-transformed rat liver was studied. An increase in the protein/DNA ratio in tumor chromatin, as compared with that of normal tissue, was established. NHP were extracted from the chromatin with 0.4 N N_2SO_4 and fractionated with two-dimensional polyacrylamide gel electrophoresis. Coomassie staining revealed more than 80 fractions with mol wt ranging 20-150 th. Some quantitative and qualitative differences in the patterns of NHP from normal and neoplastic tissues were observed. A conclusion is drawn that these differences may be related to structural and/or enzymatic components of NHP. (Author abstract) (17 Refs) (In RUS)

CYTOPLASMIC AND CONTRACTILE PROTEINS

6. FERREDOXIN REDUCTASE CATALYZES STYRENE OXIDATION TO STYRENE OXIDE.

Belvedere G, Blezza D, Cantoni L

Istituto di Richerche Farmacologiche 'Mario Negri', Via Eritrea, 62, I-20157 Milano, Italy

Experientia; 38(3):306-307 1982

The flavoprotein ferredoxin reductase catalyzed the oxidation of styrene to styrene oxide in the presence of NADPH. This reaction was inhibited by the addition of catalase and superoxide dismutase. The addition of the nonheme iron protein ferredoxin partially inhibited styrene oxidation. H_2O_2 was also able to catalyze this reaction when added to the enzyme in the absence of NADPH. (Author abstract) (20 Refs)

7. HUMAN HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE: DEMONSTRATION OF STRUCTURAL VARIANTS IN LYMPHOBLASTOID CELLS DERIVED FROM PATIENTS WITH A DEFICIENCY OF THE ENZYME.

Wilson JM, Baugher BW, Mattes PM, Daddona PE, Kelley WN
Dept. Internal Medicine, Human Purine Res. Center, Univ. Michigan Medical Sch., Ann Arbor, MI, 48109
J Clin Invest; 69(3):706-715 1982

We have explored the possibility of using cultured lymphoblasts from patients with a deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) as a source of cells for the isolation and characterization of mutant forms of the enzyme. HPRT from lymphoblasts derived from six male patients of five unrelated HPRT-deficient families was highly purified and characterized with regard to: (a) level of immunoreactive protein, (b) absolute specific activity, (c) isoelectric point, (d) migration during nondenaturing polyacrylamide gel electrophoresis, and (e) apparent subunit mol wt. These experiments were performed on small quantities of lymphoblasts using several micromethods involving protein blot analysis of crude extracts as well as isolation and characterization of enzyme labeled in culture with radioactive amino acids. The lymphoblast enzymes from four of the patients exhibited structural and functional abnormalities that were similar to the recently described abnormalities found with the highly purified erythrocyte enzymes from these same patients. In addition, a previously undescribed HPRT variant was isolated and characterized from lymphoblasts derived from two male siblings. This unique variant has been called HPRTAnn Arbor. We conclude that lymphoblastoid cell lines can be used as a source of cells for the detection, isolation and characterization of structural variants of human HPRT. (Author abstract) (32 Refs)

8. RADIORESISTANCE IN CELLS WITH HIGH CONTENT OF METALLOTHIONEIN.

Bakka A, Johnsen AS, Endresen L, Rugstad HE
Dept. Clinical Pharmacology, Rikshospitalet, Oslo, Norway
Experientia; 38(3):381-383 1982

An endogenous cytoplasmic protein, metallothionein (MT) apparently gave rise to radioresistance in two different cell lines. A dose reduction factor of 1.9 was achieved in MT-containing cells. MT accounted for a three to four-fold increase of total sulfhydryl groups in the resistant cell strains, compared to the non-resistant lines from which they were derived. The protein is rich in cysteine (30%), an amino acid known to give radioprotection when administered exogenously. Glutathione levels and cell-cycle phase distribution showed no marked difference between resistant and corresponding non-resistant cells. (Author abstract) (18 Refs)

9. CHANGES IN SOME CYTOSKELETAL PROTEINS DURING NEUROBLASTOMA CELL DIFFERENTIATION.

Edde B, Portier MM, Sahuquillo C, Jeantet C, Gros F
Laboratoire de Biochimie Cellulaire, College de France, 11, Place Marcelin Berthelot, 75231, Paris Cedex 05, France
Biochimie; 64(2):141-151 1982

After in vitro microtubule assembly of mouse neuroblastoma crude extracts, six protein species migrate in the tubulin region of two dimensional electrophoregrams. The evolution of these forms after morphological cell differentiation of the clone NIE115 shows two major modifications. Form 5 decreases drastically while Form 6 increases during neurite formation. Peptide mapping analysis reveals that Forms 5 and 6 are vimentin, a component of intermediate filaments, and beta-tubulin subunit, respectively. Sodium butyrate treatment of NIE115 cells or serum starvation of NIA103 cells, conditions blocking cell division and failing to induce morphological differentiation, prevent any modification in the relative proportion of these proteins. It is concluded that the changes in the distribution of the tubulin isoforms and vimentin are directly related to neurite formation. (Author abstract) (27 Refs)

10. DEVELOPMENTAL EXPRESSION OF MURINE EXTRA-EMBRYONIC ENDODERMAL CYTOSKELETAL PROTEINS.

Oshima RG

La Jolla Cancer Res. Foundation, 10901 North Torrey Pines Road, La Jolla, CA, 92037

J Biol Chem; 257(7):3414-3421 1982

Murine extra-embryonic endodermal cell lines derived from either teratocarcinomas or mouse embryos contain a cytoskeletal protein (Endo A) of Mr = 55,000. Endo A was immunoprecipitated from [35S]methionine-labeled lysates of three parietal endodermal cell lines, a presumptive visceral endodermal cell line, and a fetal hepatoma cell line, but not from fibroblasts, myoblasts. erythroleukemic cells, neuroblastoma cells, keratinocytes, or embryonal carcinoma cells. Embryonal carcinoma cells induced to differentiate by exposure to retinoic acid synthesized increased amounts of Endo A approx 48 hr after exposure to the inducer. Two-dimensional gel analysis of immunoprecipitated samples confirmed that Endo A is distinct from vimentin and murine keratinocyte proteins recognized by two different keratin antisera. Comparison by two-dimensional gel electrophoresis of immunoprecipitated Endo A labeled with either [35S]methionine or [32P]orthophosphate indicated that the multiple forms of Endo A resolved by isoelectric focusing were due, at least in part, to phosphorylation. Serine was identified as the phosphorylated amino acid. Endo A was the only major antigenic protein found in a parietal endodermal cell line which was recognized by a monoclonal antibody prepared by other investigators against trophoblast cytoskeletons. The results indicate that Endo A, like the previously described Endo B protein, is distinct from other cytoskeletal proteins and will be useful as a marker of the differentiation of murine embryonal carcinoma cells to extra-embryonic endoderm. (Author abstract) (46 Refs)

11. CYTOSKELETON-ASSOCIATED SURFACE GLYCOPROTEINS OF MURINE LYMPHOCYTES.

Rungger-Brandle E, Hoessli DC

Dept. Pathology, Univ. Geneva, Geneva, Switzerland

Protides Biol Fluid Proc Colloq; 29:63-66 1982

An extract of mouse (BALB/c, Balentl 5) lymphoma cells, surface-labeled with 125I, was fractionated under isotonic conditions and in the presence of a non-ionic detergent. A cytoskeletal subcompartment of light density (SCLD) was recovered which was further fractionated on a second sucrose gradient. A distinct set of labeled surface glycoproteins (including Thy-1) were identified which are associated with the SCLD. These glycoproteins may be linked to cytoskeletal elements and represent the receptor end of transmembrane linkages, within the detergent-resistant plasma membrane matrix. (8 Refs)

12. DECREASED ACTIN CONTENT OF LYMPHOCYTES FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

Stark R, Liebes LF, Nevria D, Conklyn M, Silber R
(c/o Silber) Dept. Medicine, New York Univ. Sch. Medicine, 550 First Ave., New York, NY, 10016
Blood; 59(3):536-541 1982

Actin, a major cytoskeletal protein, was quantitated in normal and chronic lymphocytic leukemia lymphocytes. The actin content of normal human blood lymphocytes was 2.2 +-0.4 mg/10(9) cells and represented 6.6% +-1.8% of the total cellular protein. A significant decrease (p less than 0.001) was noted in chronic lymphocytic leukemia lymphocytes that contained 1.4 +-0.3 mg actin/10(9) cells, constituting 4.3% +-1.1% of the total protein. Normal T and B cells did not differ in actin content. Reduced actin levels were found in the T as well as in the B lymphocytes of 'B-cell' chronic lymphocytic leukemia. The possible importance of the decreased actin level in the anomalous capping response and motility of chronic lymphocytic leukemia lymphocytes is discussed. (Author abstract) (54 Refs)

13. CYTOPLASMIC NETWORK ARRAYS DEMONSTRATED BY IMMUNOLOCALIZATION USING ANTIBODIES TO A HIGH MOLECULAR WEIGHT PROTEIN PRESENT IN CYTOSKELETAL PREPARATIONS FROM CULTURED CELLS.

Wiche G, Baker MA
Inst. Biochemistry, Univ. Vienna, 1090 Vienna, Austria
Exp Cell Res; 138(1):15-29 1982

Antisera were raised in rabbits against the two major components of intermediate filament preparations from glia-derived C6 cells, polypeptides of Mr around 300,000 and 58,000 (vimentin). These, and a third antiserum raised against microtubule proteins from hog brain, were shown to be specific for their respective immunogens. The assay employed involved the separation of components of crude cell extracts or filament preparations by SDS-polyacrylamide gel electrophoresis, and their subsequent transfer to and immobilization on nitrocellulose sheets. Cross-reacting counterparts of the immunogens were found in various cell lines, including C6, BALB/c 3T3, SV101, CHO, HeLa and PtK2 cells. In indirect immunofluorescence studies, antibodies to the high-Mr polypeptide component stained dense cytoplasmic network arrays of seemingly short, irregularly oriented fibers and lines of dots, in fibroblasts and in HeLa cells, but not in PtK2 cells. In well spread cells these networks were clearly distinguishable in morphology from the fibers decorated by antibodies to either microtubule protein or vimentin. The network arrays were resistant towards treatments with Triton X-100 and colcemid. By double immunofluorescence microscopy of single cells, using an additional antibody preparation to vimentin raised in guinea pigs, it was shown that after prolonged colcemid treatment of BALB/c 3T3 cells both, vimentin filaments and the structures stained by antibodies to the high-Mr component, accumulated in corresponding areas of the cytoplasm. The possibilities are discussed that this novel network-like structure is of the intermediate filament type and that it might function as a cross-linker of cytoplasmic - in particular cytoskeletal - elements. To signify its fluorescent localization and its possible linking role it is proposed to call the high-Mr component of intermediate filament preparations from cultured cells 'plectin'. (Author abstract) (30 Refs)

14. ALTERATION OF ACTIN CYTOSKELETON WITH TRANSFORMATION AND NEOPLASTIC PROGRESSION OF LIVER EPITHELIUM IN CULTURE.

Troianovskii SM, Bannikov GA
Lab. Mechanisms Carcinogenesis, All-Union Oncological Res. Center of USSR Acad. Medical Sciences, Moscow, USSR
Tsitologiia; 24(2):195-199 1982

Two types of changes in actin distribution pattern in the cytoskeleton were detected during the in vitro transformation of rat hepatic epithelium: reduction of inner bundles in all cells and appearance of marginal actin bundle in central cells (Type A), and reduction of marginal bundle and presence of inner bundles, preferentially oriented along the longer axis of the cells which became more polarized (Type B). Secondary redistribution of actin bundle toward Type B occurred in tumor cells arising from transformed epithelium with Type A actin pattern. High activity of gamma-glutamyl transpeptidase was found in such cells. (11 Refs) (In RUS)

15. IMMUNOHISTOCHEMICAL LOCALIZATION OF S-100 PROTEIN IN GRANULAR CELL MYOBLASTOMA.

Nakazato Y, Ishizeki J, Takahashi K, Yamaguchi H
Dept. Pathology, Gunma Univ. Sch. Medicine, 3-39-22, Showa-machi, Maebashi, Gunma, 371, Japan
Cancer; 49(8):1624-1628 1982

The presence and distribution of S-100 protein were studied in three cases of granular cell myoblastoma using the peroxidase-anti-peroxidase method. Positive immunostaining was observed in all cases. The densely brown reaction products were found both in the nuclei and in the cytoplasm of myoblastoma cells. The staining of the nuclei was more intense than that of the cytoplasm. In addition, both normal Schwann cells and tumor cells of Schwannomas were also stained with anti-S-100 antibody. This result further supports the concept of the neurogenic origin of the granular cell myoblastoma. (Author abstract) (28 Refs)

16. EXPRESSION OF MONOAMINE OXIDASE A AND B TYPES IN HYBRID CELLS OF NEUROBLASTOMA.

Nagatsu T, Nakano T, Kato T, Kano-Tanaka K, Higashida H
Lab. Cell Physiology, Dept. Life Chemistry, Graduate Sch. at Nagatsuta, Tokyo Inst. Technology, Yokohama 227, Japan
Excerpta Med Int Congr Ser; 564():279-288 1982

The usefulness of hybrid cells in studying the expression of A and B types of monoamine oxidase (MAO) activity was investigated. The MAO activity using 26.7 uM kynuramine as substrate was measured in homogenates at the stationary phase of neuroblastoma, glioma, myoblast, pituitary adenoma, and hybrid cells; the mean values of the specific activity in 2-5 samples from different cultures are listed. The highest activity, 127 picomole/min/mg protein, was observed in NCB20 hybrid cells, while neuroblastoma NB2A cells possessed no detectable activity. The other cell lines showed intermediate activities. The relative proportions of MAO A and B activities in cell homogenate were determined by measuring the sensitivity to inhibition of kynuramine oxidation by varying concentrations of clorgyline and deprenyl from 10(-10) to 10(-4) M. In nine cell lines, MAO activity was completely inhibited at 10(-7) M clorgyline. Double sigmoid inhibition curves with inhibitors were obtained in NCB20 cells. Inhibition curves obtained with five samples of G8-1 cells at various developmental stages from 0-100% in fusion (myotube formation) showed 95% type A and type B MAO activity. Thus, the ratio of type A and type B activity was unchanged through the developmental stages. The

MAO relative activities of NCB20 hybrid cells and C6BU-1 glioma cells with various substrates at concentrations previously discussed are listed. In all preparations, tyramine was most actively metabolized. Karyotype analysis of NCB20 cells is presented. The modal number of chromosomes in NCB20 cells was 86 with 2-6 Chinese hamster chromosomes. (29 Refs)

17. THE ADAPTIVE RESPONSE TO ALKYLATING AGENTS. THE REMOVAL OF O6-METHYLGUANINE FROM DNA IS NOT DEPENDENT ON DNA POLYMERASE-1.

Karran P, Stevens S, Sedgwick B
MRC Cell Mutation Unit, Univ. Sussex, Falmer, Brighton, Sussex BN1 5JH, England
Mutat Res; 104(1-3):67-73 1982

The level of O6-methylguanine (OMG) repair protein was assayed in cell-free extracts of adapted DNA-deficient (PolA) strains of Escherichia coli. Removal of OMG from the DNA of PolA strains in vivo also was examined. The ability to excise OMG from DNA was not impaired in PolA strains due to a defective induction of demethylating activity. Following adaptation, PolA strains produced normal levels of active OMG demethylating protein. Induction and operation of the OMG repair protein seemed normal. (11 Refs)

18. SWARM RAT CHONDROSARCOMA PROTEOGLYCANS. PURIFICATION OF AGGREGATES BY ZONAL CENTRIFUGATION OF PREFORMED CESIUM SULFATE GRADIENTS.

Kimata K, Kimura JH, Thonar EJ, Barrach HJ, Rennard SI, Hascall VC
Dept. Chemistry, Faculty Science, Nagoya Univ., Chikusa, Nagoya 404, Japan
J Biol Chem; 257(7):3819-3826 1982

A procedure utilizing zonal centrifugation on preformed cesium sulfate gradients is described for purifying the aggregate fraction from cartilage proteoglycan samples. The procedure permits rapid separation of aggregates from nonaggregated proteoglycans at centrifugation temperatures of 2 to 11 C with a high loading capacity. Hexuronic acid analyses are used directly on gradient fractions without interference to monitor proteoglycan distributions. For the proteoglycan fraction obtained from the Swarm rat chondrosarcoma, about 75% of the total hexuronic acid was recovered in aggregates and about 25% in nonaggregated proteoglycans. Analyses of the subfractions for (a) antigenicity with antibodies directed against either proteoglycan monomer or the purified hyaluronic acid-binding region, (b) average chondroitin sulfate chain sizes, and (c) chemical composition suggest: (1) polydispersity of aggregating monomers is primarily the result of differences in the average chondroitin sulfate chain sizes on different molecules; (2) about 15% of the aggregating monomers contain a discretely smaller core which was observed after chondroitinase digestion, possibly representing a selective breakdown product of the major population; (3) the nonaggregated proteoglycan fraction of smallest size is distinctly different from aggregating monomers; (4) the nonaggregated proteoglycans of larger size may be related to aggregating monomers but still have some compositional and antigenic differences; and (5) the extent of polydispersity of the largest nonaggregated proteoglycan fraction is similar to that of the aggregating monomers and is likewise primarily related to differences in the av chondroitin sulfate chain sized on different molecules. (39 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

19. ENZYMES OF COLLAGEN SYNTHESIS AND TYPE III PROCOLLAGEN AMINO-PROPEPTIDE IN SERUM FROM NIGERIANS WITH HEPATOCELLULAR CARCINOMA AND OTHER MALIGNANT DISEASES.

Bolarin DM, Savolainen ER, Kivirikko KI
(c/o Kivirikko) Dept. Medical Biochemistry, Univ. Oulu, SF-90220 Oulu 22, Finland
Int J Cancer; 29(4):401-405 1982

Serum immunoreactive prolyl hydroxylase protein (S-IRPH), galactosylhydroxylysyl glucosyltransferase activity (S-GGT) and the amino-terminal propeptide of Type III procollagen [S-Pro(III)-N-P] were studied in 24 patients with primary hepatocellular carcinoma, 18 with secondary liver neoplasms and 35 with other malignant diseases but no evidence of liver involvement; this latter group included 13 patients with Burkitt's lymphoma, 11 with breast cancer and 11 with other neoplasms. Control values were determined for 60 apparently healthy Nigerians. S-IRPH and S-GGT were above the upper normal limit, defined as the mean +2 SD of the controls, in all the patients with primary hepatocellular carcinoma and all but one with secondry liver neoplasms, whereas only one S-IRPH value and three S-GGT values exceeded this limit in the patients with other malignant diseases. The mean S-Pro(III)-N-P was even more elevated than S-IRPH and S-GGT in the primary and secondary liver neoplasm cases, but was also elevated in other malignant neoplasms; about one third of the patients with no evidence of liver involvement had a concentration exceeding the upper normal limit. A high correlation was found between the values for the three assays both in primary hepatocellular carcinoma and in the whole series of malignant diseases. The data suggest that primary and secondary malignant neoplasms of the liver have a high rate of collagen synthesis. The three assays may be of some value in the diagnosis of primary hepatocellular carcinoma and secondary liver involvement in other malignant diseases, and in monitoring the treatment provided. (Author abstract) (22 Refs)

20. BIOSYNTHESIS OF AN INTERSTITIAL TYPE OF COLLAGEN BY CLONED HUMAN GASTRIC CARCINOMA CELLS.

Sakakibara K, Suzuki T, Motoyama T, Watanabe H, Nagai Y
Dept. Pathobiochemical Cell Res., Inst. Medical Science, Univ. Tokyo, Shirokanedai, Minatoku, Yokyo 108, Japan
Cancer Res; 42(5):2019-2027 1982

In an attempt to elucidate the role played by neoplastic epithelial cells in the formation of stromal collagen, the synthesis of collagen by two cloned human gastric carcinoma cell lines was studied. The presence of antigenicity of procollagen alpha1(I) chain in the cytoplasms of both carcinoma cell lines growing in culture was demonstrated by an immunocytochemical technique using specific antibodies. After denaturation of the radiolabeled collagenous proteins extracted from the combined cells and culture media, two components comigrating with authentic alpha1(I) and alpha2 chains on sodium dodecyl sulfate:polyacrylamide gel electrophoresis were found. Electrophoretogram analysis revealed further a dense band slightly slower than the alpha1(I) chain, most likely representing alpha1(I) trimer. These radioactive components disappeared after exposure of the samples to bacterial collagenase. The relative activity of collagen synthesis determined by using purified collagenase was slightly higher than that of fibroblasts derived from human synovial membrane in culture. The same antibodies to procollagen alpha1(I) chain also labeled the cytoplasms of carcinoma cells, and extracellular matrix of the tumors developed after transplantation of one of the cell lines into the nude mice. Our data indicate that both human gastric carcinoma cell lines synthesize type 1 collagen in vivo as well as in vitro and suggest that carcinoma cells may play an active role in the formation of stromal collagen in most human carcinomas. (Author abstract) (41 Refs)

21. IMMUNOFLUORESCENT LOCALIZATION OF TYPE IV COLLAGEN AND LAMININ IN HUMAN SKIN AND ITS APPLICATION IN JUNCTIONAL ZONE PATHOLOGY.

Weber L, Krieg T, Muller PK, Kirsch E, Timpl R
Abteilung Dermatologie, Universitat Ulm, Oberer Eselsberg 40, D-7900 Ulm, W. Germany
Br J Dermatol; 106(3):267-273 1982

Purified antibodies against Type IV collagen and laminin were used to localize basement membranes by indirect immunofluorescence in various anatomical regions of normal and diseased human skin. The two proteins showed extensive codistribution. A continuous linear staining was found along the epidermal-dermal junction and around hair follicles, sebaceous gland acini and small capillaries. The same proteins also surrounded individual cells such as those found in vessels, hair erector muscles and subcutaneous tissue. Blister formation in bullous pemphigoid left Type IV collagen and laminin on the floor of the blister, while the bullous pemphigoid antigen as detected by human autoantibodies was found on both sides of the blister. In solid basal cell carcinoma a strong staining was found around all tumor islands as well as focally within the cell clusters. This suggests that the tumor cells produce these basement membrane proteins but have lost, at least in part, control of polar deposition. (Author abstract) (28 Refs)

22. PROTEASE RESISTANCE AND CONFORMATION OF LAMININ.

Ott U, Odermatt E, Engel J, Furthmayr H, Timpl R
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Eur J Biochem; 123(1):63-72 1982

Laminin, obtained from a tumor basement membrane, was treated with neutral proteases (trypsin, chymotrypsin, elastase, subtilisin, Staphylococcus aureus protease), which all produced similar fragment patterns upon prolonged digestion. The patterns were different from those obtained for fibronectin, which showed a much higher susceptibility to proteolysis by the same enzymes. Four large fragments could be purified which accounted together for more than half of the mass of laminin. They were found to differ in size, amino acid composition, spectral properties and antigenicity. The largest fragment 1(Mr 260,000-300,000) was rich in half-cystine (120 residues/1,000) and showed a circular dichroism spectrum indicating the absence of alpha helix and beta structure. Fragment 3 (Mr = 50,000) possessed beta structure and was able to bind onto heparin-Sepharose. Fragments 2 (Mr = 50,000) and 4 (Mr = 75,000) were related structures and their relative yields depended on the protease used. They showed spectra similar to those of fragment 1. Electron microscopy revealed that fragment 1 consists of three rodlike elements (length 26 nm) connected to each other at one end. The other fragments appeared as globules (fragment 3), short rods (fragment 2) or globules connected to a short rod (fragment 4). Data obtained from limited proteolysis indicated that fragment 1 and 4 (or 2) are in close proximity to each other in the three short arms of laminin, which in its intact form has the shape of an asymmetric cross. The long arm appeared to be readily degraded by proteases. (Author abstract) (29 Refs)

23. IMMUNOGLOBULIN M AUTOANTIBODY TO VIMENTIN INTERMEDIATE FILAMENTS.

Senecal JL, Rothfield NF, Oliver JM

Div. Rheumatic Diseases, Dept. Medicine, Univ. Connecticut Health Center, Farmington, CT, 06032

J Clin Invest; 69(3):716-721 1982

Serum from a patient with the CREST Syndrome and systemic lupus erythematosus contained an IgM antibody that reacted at dilutions up to 1:800 with a fibrous cytoplasmic network in several epithelioid and fibroblastic cell lines. The antibody was shown by immunofluorescence microscopy to label a specific subset of cytoskeletal polymers, the intermediate filaments. The reactive antigen from this biochemically heterogeneous group of filaments was established as the 58,000-mol wt protein, vimentin: (a) the patient's serum reacts with a range of cell lines that contain intermediate filaments composed of vimentin, but not with cells whose intermediate filaments are composed of different protein subunits; (b) in PTK2 epithelioid cells the serum reacts with the class of filaments that coils around the nucleus after colchicine treatment (vimentin) and not with the filaments that remain dispersed after colchicine (pre-keratin); and (c) the component of reactive cells that combines with the serum is shown by immunoelectrophoresis to be a 58,000-mol wt protein antigen. A similar antibody that binds intermediate filaments of PTK2 cells was encountered at lower titer in some sera from other patients with connective tissue diseases and in control sera. Previous routine antinuclear antibody assays using mouse liver or commercially prepared HEp-2 cells have failed to reveal anticytoskeletal antibodies in patient sera, perhaps due to inadequate presentation or preservation of cytoplasmic antigens. (Author abstract) (25 Refs)

24. ELASTIN DEGRADATION BY PROTEASES FROM CULTURED HUMAN BREAST CANCER CELLS.

Kao RT, Wong M, Stern R

Dept. Pathology, Univ. California San Francisco, San Francisco, CA, 94143

Biochem Biophys Res Commun; 105(1):383-389 1982

The human breast tumor cell lines ZR75-1, ZR75-30, ZR75-31A, and MFC-7 were found to exhibit elastinolytic activity in culture. DEAE-cellulose chromatography was used to separate the elastase-like activity of ZR75-31A cells into three different proteases; two were identified as serine proteases and one a metalloprotease based on inhibition studies with protease inhibitors. Each existed in an inactive form and required trypsin pretreatment for activity. The enzymes may be involved in modulation and turnover of the elastin-containing desmoplastic reaction found in most human breast cancers. (14 Refs)

25. BIOCHEMICAL PROPERTIES OF THE HIGH-MOLECULAR-WEIGHT GLYCOPEPTIDES RELEASED FROM THE CELL SURFACE OF HUMAN TERATOCARCINOMA CELLS.

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Cancer Res; 42(5):1749-1752 1982

The present study deals with the biochemical properties of high-molecular-weight glycopeptides isolated from the surface of human teratocarcinoma cells. This cell surface material released by mild trypsin digestion from galactose-labeled human teratocarcinoma cells, Tera I and PA1, was digested extensively with pronase. Most of the resulting glycopeptides were large and were excluded from a Sephadex G-50 column. The properties of these large cell surface glycopeptides isolated from Tera I cells have been examined in detail. It is clear from these experiments that they are neither acidic mucopolysaccharides nor mucin-type glycopeptides with short oligosaccharide chains. Although the glycopeptides are hardly hydrolyzed by beta-galactosidase even after prior digestion with neuraminidase, around 30% of the glycopeptides are depolymerized by treatment with endo-beta-galactosidase from Escherichia freundii. The large cell surface glycopeptides from Tera I cells therefore appear to be very similar to the large glycopeptides seen on mouse embryonal carcinoma cells, which have core structures composed of galactose and N-acetylglucosamine. Like the mouse cell glycopeptides, a fraction of the large glycopeptides from these human cells bind to agarose-conjugated fucose-binding proteins and peanut agglutinin. (Author abstract) (14 Refs)

26. PURIFICATION OF GLYCOPROTEIN FROM 'CONDITIONED MEDIUM' OF SPONTANEOUSLY MALIGNANT TRANSFORMED FIBROBLAST CELLS; CELL-SPREADING EFFECT OF THIS GLYCOPROTEIN ON SO-CALLED RETICULUM CELLS CULTURED IN SERUM-FREE MEDIUM.

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Dept. Pathology, Faculty Medicine, Kyoto Univ., Kyoto, Japan

Cell Mol Biol; 27(6):709-717 1981

Mouse fibroblast cells (m cells) and so-called reticulum cells (MR cells and SP cells) transformed into malignant cells in vitro, were transferred to a serum-free culture medium, and the morphological and functional differences of these two kinds of cells were studied. We found that the fibroblast cells produce a unique glycoprotein which has the biological function of inducing cell-spreading of so-called reticulum cells on the substratum in the serum-free culture medium. This glycoprotein was purified from the conditioned medium of the transformed fibroblast cells. The mol wt of this protein is about 230-250 x 10(3) daltons. The so-called reticulum cells themselves do not produce detectable amounts of this protein. Specific antiserum against this glycoprotein shows a single precipitation line against not only this glycoprotein, but also against serum protein. This antiserum inhibits the cell-spreading inducing activity of this glycoprotein in vitro. Immunofluorescent microscopy shows that this glycoprotein exists on the cell surface and in the cytoplasm of normal and some transformed fibroblast cells. Undifferentiated fibrosarcoma cells, however, do not produce this glycoprotein in vitro. (Author abstract) (24 Refs)

27. A GROWTH FACTOR PRODUCED BY WEHI-3 CELLS FOR MURINE HIGH PROLIFERATIVE POTENTIAL GM-PROGENITOR COLONY FORMING CELLS.

McNiece IK, Bradley TR, Kriegler AB, Hodgson GS

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Cell Biol Int Rep; 6(3):243-251 1982

Preparation and properties of 'synergistic activity' (SA), a hemopoietic growth factor, from media conditioned by a myelomonocytic leukemic cell line (WEHI-3) are described. SA was prepared in high yield by conditioning serum-free medium with WEHI-3 cells that had been grown in vivo (BALB/c mice). SA was shown not to be stored in WEHI-3 cells. Storage at 4C and-20 C did not affect the activity. SA was stable between pH 2.0 and 10.6 and to heating for 1 hr at less than or equal to 50 C, but was unstable to storage at room temperature, trypsin digestion, 8 M urea with and without 4 milliM mercaptoethanol (ME) at pH 8.0, and to ME alone. Instability in the presence of ME indicated the presence of a disulfide bond, while inactivation by trypsin suggested that SA is a protein. The apparent mol wt was 9,000-25,000, and the isoelectric point was at pH 4.0-6.5. SA did not bind to concanavalin-A Sepharose. Morphologic studies showed that all colonies contained virtually pure populations of macrophages. (10 Refs)

28. ARGININE CONTENT IN SERUM PROTEIN FRACTIONS OF MICE WITH MAMMARY ADENOCARCINOMA.

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Dept. Biochemistry, Inst. Biochemistry and Medical Analytics, Medical Acad., 15-230 Bialstok 8, Poland

Arch Geschwulstforsch; 52(3):175-179 1982

Arginine content and arginine-rich low mol wt peptides were assayed in blood serum of C3H female mice with mammary adenocarcinoma. Concentration of total serum proteins and albumin fraction of mice with 4-wk old tumors was lower than in the control group. Simultaneously, significant increases of total arginine, arginine of albumin fraction, and of alpha1, beta, and gamma globulin fractions were observed. Chromatographic separation of amino acids and peptides of deproteinized sera from control and neoplastic animals was demonstrated. Apparently arginine-rich basic peptides were bound with particular fractions of serum proteins. Thus, increased arginine content in particular protein fractions of sera is a common feature in various kinds of neoplastic diseases. (8 Refs)

29. DEPOSITION OF BASEMENT MEMBRANE PROTEINS IN ATTACHMENT AND NEURITE FORMATION OF CULTURED MURINE C-1300 NEUROBLASTOMA CELLS.

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J Cell Biochem; 18(1):25-35 1982

The deposition of basement membrane (BM) glycoproteins during the processes of substrate attachment and cell differentiation was studied in cultures of C-1300 mouse neuroblastoma cells (clone NB41A3). Cell differentiation was induced by sparse plating and the addition of dibutyryl cyclic AMP (dbcAMP: 1 milliM) to the medium. Indirect immunofluorescence microscopy disclosed a typical perinuclear cytoplasmic staining for BM antigens in both differentiated and undifferentiated cells. No surface fluorescence was detected in freshly seeded cells in suspension, but punctate deposits of laminin were detected on the ventral surfaces of the cells within 2 hr after plating. In sparsely seeded cultures of undifferentiated C-1300 cells, extracellular laminin deposits were detected only under the primary attaching cells, not the daughter cells in the clonal colonies. In dbcAMP-exposed cultures, laminin deposition was detected in association with the growing cytoplasmic extensions of the newly formed neurites. The secreted proteins of cultured differentiated and undifferentiated neuroblastoma cells showed markedly different patterns of fucosylation of the high-mol wt proteins. No deposits of fibronectin or Type IV collagen were found under the attaching and a differentiating cells. It is concluded that cultured C-1300 cells can synthesize, secrete, and deposit laminin. The distribution of laminin during cell attachment and neurite extension suggests that laminin may be involved in cell-to-substrate interactions in cultured C-1300 neuroblastoma cells. (53 Refs)

30. ANTIGENICITIES OF MUCINS - THEIR RELEVANCE TO TUMOUR ASSOCIATED AND STAGE SPECIFIC EMBRYONIC ANTIGENS.

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Applied Immunochemistry Res. Group, Div. Communicable Diseases, Clinical Res. Centre, Harrow, Middlesex, England
Adv Exp Med Biol; 144:29-37 1982

Differentiation antigens expressed in secreted glycoproteins are reviewed. Secreted glycoproteins have provided fundamental information on the nature of the major blood group antigens and their precursors. Blood group branched (I) and linear antigens are expressed on type 2 precursor chains; the expression of I antigen of 'Ma type' in gastric mucins is genetically determined. Changes in antigenicity of gastric mucosal glycoproteins associated with cancer are discussed. Mucins could serve as valuable sources of oligosaccharide haptens of differentiation antigens. (17 Refs)

BINDING/ RECEPTOR PROTEINS

31. RECEPTORS FOR REGULATORY PEPTIDES IN EPITHELIAL GUT CANCER.

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Protides Biol Fluid Proc Colloq; 29:595-598 1982

Studies on receptors for regulatory peptides, such as insulin and vasoactive intestinal peptide (VIP) in transformed gut epithelial cell lines from humans are reviewed. The coupling of insulin and VIP with their specific receptors triggers regulatory events: insulin regulates long-term effects (protein, RNA, and DNA synthesis), while VIP regulates events leading to glycogenolysis by activation of adenylate cyclase, cyclic AMP-dependent protein kinases, and phosphorylase a. Insulin is effective at very low concentration (10(-10) M). It was demonstrated that transformed cells of the digestive tract possess specific receptors for regulatory peptides which make them sensitive to their neuroendocrine environment. The insulin effect was not uniform; even in the same type of cell, the effect on growth can vary according to the batch of serum used. These receptors are coupled to a biological activity, the magnitude of which varies according to the cell line. (30 Refs)

32. DESENSITIZATION OF TUMOUR LEYDIG CELLS BY LUTROPIN: EVIDENCE FOR UNCOUPLING OF THE LUTROPIN RECEPTOR FROM THE GUANINE NUCLEOTIDE-BINDING PROTEIN.

Dix CJ, Schumacher M, Cooke BA
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Biochem J; 202(3):739-745 1982

Purified rat Leydig tumors cells were pretreated with lutropin and the effect on the subsequent response to lutropin was determined. Maximal cyclic AMP production was achieved with the same concentration of lutropin in control and lutropin-pretreated cells; however, the maximum stimulated level in pretreated cells was only 30% of controls. The sensitivity to lutropin was decreased in lutropin-pretreated cells, as was the rate of maximal cyclic AMP production. However, cholera-toxin-stimulated cyclic AMP production was not decreased by lutropin pretreatment, and a potentiation was seen at all time points studied. Pre-incubation with lutropin caused a decrease in specific 125I-labeled human choriogonadotropin binding; however, this decrease was abolished if the cells were washed under acidic conditions, indicating that occupation but not loss of the lutropin receptors had taken place. The effect of pretreating the cells with lutropin on adenylate cyclase activity in purified plasma membranes was also investigated. In plasma membranes from control cells both guanosine 5'-[beta-gamma-imido]triphosphate [p(NH)ppG] plus lutropin and NaF plus lutropin caused a 50-60-fold linear increase in cyclic AMP production over 40 min compared with 15-fold with p(NH)ppG and 6-fold with lutropin alone. In plasma membranes isolated from lutropin-treated cells the NaF-plus-lutropin- and the p(NH)ppG-stimulated cyclic AMP production rates were unchanged but no effect of lutropin could be demonstrated with or without added p(NH)ppG. In contrast the plasma membranes from dibutyryl cyclic AMP-treated cells had similar cyclic AMP production rates to control cells with all stimulants studied. The present evidence obtained from studies both with intact cells and with isolated plasma membranes indicates that the initial lutropin-induced desensitization of the rat Leydig tumor cell is due to a lesion in the hormone-receptor coupling to the guanine nucleotide regulatory protein. This process is apparently not regulated by cyclic AMP. (33 Refs)

33. FC RECEPTORS OF MOUSE CELL LINES. II. IGG BINDING SPECIFICITY AND IDENTIFICATION OF THE FC RECEPTOR ON A LYMPHOID LEUKEMIA.

Lane BC, Bricker MD, Cooper SM
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J Immunol; 128(4):1825-1831 1982

We determined the binding specificity of the Fc receptors on the murine lymphoid leukemia, L1210, which bears several surface antigens characteristic of B cells and B cell precursors. L1210 possesses an Fc receptor that binds monomer IgG of the IgG1, IgG2a, and IgG2b subclasses with low affinity, whereas aggregated IgG of these subclasses is bound comparatively well. There was no detectable binding of monomer or aggregated IgG3. Although direct binding of monomer IgG was difficult to demonstrate, monomer inhibition of aggregate binding was found at monomer concentrations in excess of 0.2 mg/ml. There was cross-competition between monomers and aggregates of all three subclasses, indicating that all were binding to the same Fc receptor. Isolation and characterization of the Fc receptor of L1210 was accomplished by affinity chromatography of the radiolabeled cell lysate over columns of Sepharose 4B coupled to various IgG and control proteins. A single protein, with an approx mw of 60,000, was found to specifically bind to the Fc portion of IgG. This protein was demonstrated in the acetic acid and SDS eluates from columns coupled to monomer, aggregated, or antigen-complexed IgG, but was not seen in eluates from F(ab')2 columns or from eluates of IgG columns that received the lysates of Fc receptor-negative cells. The 60-kd Fc binding protein was also demonstrated in the SDS eluates from affinity columns of Sepharose 4B coupled to myeloma proteins of mouse IgG1, IgG2a, and IgG2b subclasses. These studies indicate that in contrast to mouse macrophage-like cell lines that possess separate IgG subclass specific Fc receptors, L1210, a murine leukemia of possible B cell lineage, possesses a single Fc receptor with distinct binding and structural characteristics. (Author abstract) (40 Refs)

34. OPIOID PEPTIDES INDUCE REDUCTION OF ENKEPHALIN RECEPTORS IN CULTURED NEUROBLASTOMA CELLS.

Chang KJ, Eckel RW, Blanchard SG
Molecular Biology Dept., Wellcome Res. Laboratories, Research Triangle Park, NC, 27709
Nature; 296(5856):446-448 1982

After several hr of exposure of monolayer cultures of mouse neuroblastoma N4TG1 cells to enkephalin, the number of enkephalin receptors measured subsequently in the extensively washed membrane preparations was greatly reduced. The reduction in receptor number seemed to be selective for opioid peptides and was antagonized by a variety of opiate agonists and antagonists. The observations are discussed in relation to the phenomenon of opioid-induced desensitization. (22 Refs)

35. THE BIOCHEMICAL AND GENETIC BASIS FOR THE MICROHETEROGENEITY OF HUMAN R-TYPE VITAMIN B12 BINDING PROTEINS.

Yang SY, Coleman PS, Dupont B
Dept. Immunogenetics, Sydney Farber Cancer Res. Inst., 44 Binney St., Boston, MA, 02115
Blood; 59(4):747-755 1982

R-type vitamin B12 binding proteins (R proteins) from human granulocytes, erythrocytes, plasma, and other body fluids were characterized by isoprotein banding patterns on autoradiograms after resolution via thin-layer polyacrylamide isoelectric focusing (IEF) gel electrophoresis. R proteins obtained from various tissue sources in a given individual show tissue-specific electrophoretic patterns. The desialated R proteins obtained following in vitro treatment with neuraminidase are, however, the same for any given individual and do not show tissue specificity. The differences seen in native R proteins (ie, transcobalamin I, III, and others) obtained from different tissues are due to variations only in the sialic acid content. Granulocytes from patients with chronic myelogenous leukemia (CML) contain both TC I and TC III, and these R proteins can be released in vitro by lithium stimulation. Normal granulocytes contain only TC III. Differences in desialated R proteins from individual to individual are due to a genetic polymorphism controlled by a single genetic locus (designated TCR) with two alleles, 1 and 2, which are found to be codominantly expressed in heterozygous individuals. The allelic variants of the desialated R proteins found in different blood cells and body fluids are controlled by only one genetic locus. (Author abstract) (34 Refs)

MEMBRANE/ SURFACE PROTEINS

36. TWO MONOCLONAL ANTIPLATELET ANTIBODIES AS MARKERS OF HUMAN MEGAKARYOCYTE MATURATION: IMMUNOFLUORESCENT STAINING AND PLATELET PEROXIDASE DETECTION IN MEGAKARYOCYTE COLONIES AND IN IN VIVO CELLS FROM NORMAL AND LEUKEMIC PATIENTS.

Vainchenker W, Deschamps JF, Bastin JM, Guichard J, Titeux M, Breton-Gorius J, McMichael AJ
Hopital Henri Mondor, Unite INSERM U.91, 94010 Creteil, France
Blood; 59(3):514-521 1982

Two monoclonal anti-human platelet antibodies have been used in an immunofluorescent assay to study megakaryocyte maturation. The two antibodies were specific for platelets and megakaryocytes. AN 51 recognizes an antigen on platelet glycoprotein Ib, the antigen detected by J 15 is on the glycoprotein IIb/IIIa complex, since it does not bind to platelets from patients with Glanzmann's thrombasthenia. AN 51 did not stain small cells of normal bone marrow, but it labeled most of the megakaryocytes and all of the platelets. In megakaryocyte colonies, megakaryocytes that had reached full maturity (at day 12 of culture) were stained specifically. AN 51 also labeled micromegakaryocytes (small mature megakaryocytes) in fetal or neonatal cultures and in leukemias, but could not be applied to identification of most leukemia promegakaryoblasts. In contrast, J 15 labeled all megakaryocytes in bone marrow as well as some rare small cells. These cells appear to represent an early cell of the megakaryocyte lineage, since their number was increased in autoimmune thrombocytopenia and they were present as early as the 6th day of culture of megakaryocyte colonies. The size of these cells progressively increased as the culture aged, probably due to endoreplication. In the bone marrow of three patients with leukemias in which promegakaryoblasts were identified ultrastructurally by the presence of platelet peroxidase, J 15 labeled blast cells. Thus, glycoprotein Ib is progressively expressed during megakaryocyte maturation, but its expression is independent of megakaryocyte ploidy, while the glycoprotein IIb/IIIa complex is an early antigenic marker of megakaryocyte maturation that may be useful in isolation of megakaryocyte precursors and diagnosis of megakaryoblastic leukemia. (Author abstract) (32 Refs)

37. DISTRIBUTION OF A 100K DALTON GLUCOSE-REGULATED CELL SURFACE PROTEIN IN MAMMALIAN CELL CULTURES AND SECTIONED TISSUES.

McCormick PJ, Millis AJ, Babiarz B
Lab. Developmental Genetics, Sloan-Kettering Inst. Cancer Res., New York, NY, 10021
Exp Cell Res; 138(1):63-72 1982

Previously, we described a glucose-regulated cell surface glycoprotein (100K-GRP) which is present on the surfaces of non-transformed human fibroblasts. The 100K-GRP is released into the culture media in a soluble form. In this report, we utilize an antibody raised to the 100K-GRP and indirect immunofluorescence to localize the 100 K-GRP on human and mouse cells in vitro, and on mouse tissues in vivo. On cultured cells, the 100K-GRP is associated with fibers in the pericellular matrix. Examination of sectioned mouse tissues reveals that the 100K-GRP is present in a wide variety of tissues. However, in contrast to fibronectin, it is not found in extracellular structures such as basement membranes. In vivo, the 100K-GRP is only found in close association with the surfaces of individual cells. (Author abstract) (20 Refs)

38. THE COOPERATIVE ROLE OF THE TRANSFORMATION-SENSITIVE GLYCOPROTEINS, GP140 AND FIBRONECTIN, IN CELL ATTACHMENT AND SPREADING.

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Biochemical Oncology, Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Biol Chem; 257(6):3249-3257 1982

The detergent-insoluble matrix of cultured human fibroblasts contains cytoskeletal and nuclear components, as well as two major, noncollagenous glycoproteins, fibronectin and GP140. These glycoproteins are stabilized by extensive intermolecular disulfide bonding. GP140 was partially purified, and a higher yield of GP140 could be obtained under reducing conditions by extraction with urea-dithiothreitol followed by molecular sieve chromatography. The purified GP140 contained mannose, galactose, and N-acetylglucosamine residues. Amino acid analysis of GP140 detected periodate-sensitive residues, as well as hydroxylproline. These hydroxylated amino acids are major components of collagens and collagen-like proteins. The GP140 isolated under nonreducing conditions was found to induce stable cell attachment and cell spreading when coated on plastic surfaces. The cell attachment could not be inhibited with affinity purified anti-fibronectin antibodies. However, trypsinization of cells under conditions that removed surface fibronectin reduced the ability of the cells to bind to the GP140-coated surface. Metabolic labeling of cells with radioactive glucosamine during 1-hr cell attachment experiments incorporated label into both fibronectin and GP140, indicating that the cells rapidly glycosylate both fibronectin and GP140 and incorporate them into the stable detergent-insoluble matrix. Long term labeling and chase experiments indicated that fibronectin and GP140 in the matrix are subject to very slow turnover. Neither fibronectin nor GP140 were detectable in the detergent-insoluble matrix of SV40-transformed human fibroblasts by either metabolic or cell surface labeling. These results are consistent with the conclusion that fibronectin and GP140 may function in a cooperative manner in cell adhesion and spreading. (70 Refs)

39. INTEGRAL MEMBRANE GLYCOPROTEINS RELATED TO CELL-SUBSTRATUM ADHESION IN MAMMALIAN CELLS.

Damsky CH, Knudsen KA, Buck CA
Wistar Inst. Anatomy and Biology, Philadelphia, PA, 19104
J Cell Biochem; 18(1):1-13 1982

Goat antisera were raised against surface membrane-derived material from baby hamster kidney cells (BHK21/C13), a transformed line derived from cell line (C13/B4), and mouse mammary tumor epithelial cells (BALB/c MT2). The antisera to the hamster cell lines (anti-M) and the MT2 cells (anti-GP) disrupted cell-substrate adhesions in cultures of their respective cell types. An antibody blocking assay was used to isolate adhesion-related glycoproteins (GP) from non-ionic detergent (Nonidet NP40) extracts of each cell type. In each case, the product was a restricted population of GPs (mol wt about 120,000-160,000). Each GP fraction blocked the disruption of adhesion induced by the goat antiserum on either cell type (hamster kidney or mouse mammary tumor). The antisera were capable of disrupting cell-substratum adhesion of a large number of cell types and species sources. Antibody-blocking activity was detected in partially purified extracts of a variety of cultured cell types. In addition to similar substrate-associated GPs (eg, fibronectin) and cytoskeleton-associated proteins (eg, alpha-actinin and vinculin), cells from different tissues and different species apparently contained a relatively conserved class of integral membrane GPs involved in cell-substrate adhesion. (29 Refs)

40. MACROPHAGE SURFACE COMPONENT GP160: SENSITIVITY TO PLASMIN AND OTHER PROTEASES.

Remold-O'Donnell E, Lewandrowski K
Center Blood Res. and Dept. Biological Chemistry, Harvard Medical Sch., Boston, MA
J Immunol; 128(4):1541-1544 1982

The correlation between activation of macrophages and increased secretion of plasminogen activator suggests that macrophages are exposed to the protease plasmin. Incubation of 125I-labeled, caseinate-elicited guinea pig peritoneal macrophages with plasmin cleaves surface protein, gp160, characterized previously by its sensitivity to trypsin. The gp160 fragments produced by plasmin (fr85 and fr71), which remain disulfide-bonded to the membrane, comigrate with the fragments produced by trypsin, indicating close or identical cleavage sites. No other detectable 125I-labeled surface component is cleaved by plasmin. Neither gp160 nor any other detectable 125I-labeled surface component was cleaved by a series of other proteases associated with inflammation including thrombin, collagenase, pancreatic elastase, leukocyte elastase, cathepsin G, and urokinase. Analysis with the use of homogeneous plasmin from guinea pig plasma shows that concentrations as low as 50 ug/ml cause measurable cleavage of gp160 in 30 min. (Author abstract) (23 Refs)

41. FIBRONECTIN.

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Int J Dermatol; 21(2):68-74 1982

Structural, chemical, and functional aspects of fibronectin (FN) in the human body are reviewed, with emphasis on the glycoprotein's role in dermatological problems of structural or immunological origin. FN in transformed cell lines and in the skin is discussed; immunofluorescence patterns of healthy and diseased skin are shown. (25 Refs)

42. ENHANCED NEURAMINIDASE-RESISTANT SIALIC ACID CONTENT IN THE SURFACE GLYCOPEPTIDES OF HUMAN LEUKAEMIC HAIRY CELLS.

Van der Hofstad GA, Van Rymenant ME
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Protides Biol Fluid Proc Colloq; 29:75-78 1982

Previously, a difference was noted in the elution profiles obtained after gel filtration of fucose-containing surface glycopeptides (GLC) from leukemic cells of hairy cell (HC) leukemia patients compared to those from control cells. HC preparations were isolated either from heparinized peripheral blood or from spleen tissue of HCL patients and labeled metabolically with 3H-fucose. Control lymphocytes isolated from healthy volunteers were labeled with 14C-fucose. The two preparations were cochromatographed, and as previously reported, the HC GLC eluted faster from the column and were enriched in the main peak of GLC as compared to the control. However, the shift to higher mol wt of the HC vs control GLC was not abolished after neuraminidase (NR) treatment as in other tumor systems, but instead an enhancement of the shift was found. Furthermore the main peak of radioactivity was relatively decreased in the HC elution compared with untreated GLC. In a parallel experiment, when part of the material was hydrolyzed mildly with HCl (0.01 N for 1.5 hr at 80 C), the difference between HC and control elution profiles was almost completely abolished. It is concluded that the change observed in untreated GLC is determined in large part by an enhanced amount of NR-resistant sialyl residues in the HC glycoproteins. (7 Refs)

43. MURINE CELL SURFACE GLYCOPROTEINS. IDENTIFICATION, PURIFICATION, AND CHARACTERIZATION OF A MAJOR GLYCOSYLATED COMPONENT OF 110,000 DALTONS BY USE OF A MONOCLONAL ANTIBODY.

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(c/o August) Dept. Pharmacology and Experimental Therapeutics, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
J Biol Chem; 257(7):3970-3977 1982

A 110,000-dalton plasma membrane glycoprotein of mouse cells has been identified, purified, and characterized by use of a xenogeneic monoclonal antibody. The glycoprotein was a major component of the NIH/3T3 cells surface. It contained 15% of [3H]glucosamine incorporated into cell proteins and was composed of at least 16 isomorphic variants with apparent mol wt from 100,000 to 120,000 and isoelectric points between 6.5 and 8.1. The glycoprotein constituted 0.1% of total cell protein, as measured by the yield of purified protein, and there were over 10(6) antibody-binding sites/cell. Immunoprecipitation from pulse-chase labeled cells showed that the 110,000-dalton glycoprotein was initially synthesized as a 92,000-dalton microsomal polypeptide which gradually was converted to the mature surface form. The turnover time of the surface form was at least 20 hr. The antigenic determinant recognized by the monoclonal antibody was species-specific and nonpolymorphic. It was present in high concentration on most dividing murine cells in culture, whereas the concentration in normal mouse tissues differed widely. Among lymphoid tissues, antigen concentration was enriched in bone marrow as compared to spleen and thymus. Among nonlymphoid tissues, antigen concentration was 10-fold greater in kidney than in brain, liver, and skeletal muscle. The glycoprotein was purified without loss of antigenic activity by antibody affinity chromatography. The single step procedure yielded 3 mg of pure glycoprotein from 3 g of crude cell extract. The purification and characterization of this major membrane protein provide a basis for further study of its cell surface structure and function. (Author abstract) (50 Refs)

44. GLYCOPROTEINS FROM TERATOCARCINOMAS AND ORGANS OF ADULT MICE: ANALYSIS BY AFFINITY CHROMATOGRAPHY ON LECTIN-CONJUGATED AGAROSE.

Ozawa M, Fijiyama J, Furukawa T, Sato K, Noguchi T, Muramatsu T
Dept. Biochemistry, Kagoshima Univ. Sch. Medicine, Usukicho, Kagoshima 890, Japan
Dev Growth Differ; 23(5):515-522 1981

Some general properties of glycoproteins (GLP) from teratocarcinomas were studied and compared with the properties of GLP from adult organs. GLP from the particulate fractions of four teratocarcinomas (OTT 6050, F9, STT-2, OTT 10A) and testis, liver, kidney, spleen, brain, small intestine, and muscle of adult mice were analyzed by sodium dodecyl sulfate gel electrophoresis after solubilization with detergents and isolation by affinity chromatography using five lectins with different specificities. The lectins employed were concanavalin A, wheat germ agglutinin (WGA), Ricinus communis agglutinin (RCA), peanut agglutinin (PNA), and Dolichos biflorus agglutinin. All the teratocarcinomas gave an intense band with an apparent mol wt of 145,000 (145K). This same band was present in testis but not the other organs. Teratocarcinoma OTT 6050, which was the only one in ascitic form, gave a distinctive high mol wt band of greater than 245K, and in general gave profiles similar to other teratocarcinomas. GLP with mol wt greater than 240,000 daltons were present in RCA, PNA and WGA receptors of OTT 6050 tumors, but not in the other teratocarcinomas. The WGA receptors of all adult organs except muscle and small intestine gave more intense bands those those of the teratocarcinomas. (27 Refs)

45. CHARACTERIZATION OF ALPHA-TISSUE ANTIGEN (ALPHATA), A MEMBRANE GLYCOPROTEIN OF CANCER CELLS.

Yachi A, Kawaharada M, Akahonai Y, Imai K, Wada T
Dept. Internal Medicine (Section 1), Sapporo Medical Coll., Sapporo, Japan
Protides Biol Fluid Proc Colloq; 29:93-96 1982

An immunochemical, biochemical and immunohistological study is presented of alpha-tissue antigen (aAT) isolated from perchloric acid soluble extracts of cancer tissue. The aAT is a glycoprotein with a mol wt of 41,000 daltons, an isoelectric pH of 3.4, and a sugar content of 10%. It is antigenically distinct from membrane associated tissular autoantigen of van Kleist as well as from other tumor associated antigens including carcinoembryonic antigen, alpha-fetoprotein and ferritin. While not cancer specific, immunofluorescent and rosette formation studies indicate aAT may be membrane-associated on cancer cells. (4 Refs)

46. TISSUE DISTRIBUTION AND MOLECULAR PROFILE OF A DIFFERENTIATION ANTIGEN DETECTED BY A MONOCLONAL ANTIBODY (345.134S) PRODUCED AGAINST HUMAN MELANOMA CELLS.

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Cancer Immunol Immunother; 12(2):159-166 1982

The mouse IgG2a monoclonal antibody (MoAb) 345.134S secreted by a hybridoma derived from a mouse immunized with cultured human melanoma cells was examined in relation to the structure of the target antigen, the distribution of the antigen in normal and malignant tissues, and the expression of the antigen on cultured cells and its ability to function as a target of immune lysis. The structure detected by the MoAb 345.134S is composed of an 85 kilodalton (KD) glycopolypeptide linked by disulfide bridges with a 30 KD polypeptide carrying little or no carbohydrate. The 115 KD antigen appears to be peripheral rather than integral to the plasma membrane. Indirect immunofluorescence studies showed that the reactivity pattern of normal tissues with MoAb 345.134S does not correlate with their embryological origin. The majority of normal tissues gave only weak staining with MoAb 345.134S. All the types of tumors tested, except liver adenocarcinomas, reacted strongly with MoAb 345.134S, although there were some cases within each tumor group that were negative. MoAb 345.134S reacted in a radioimmunometric assay with a variety of cell lines, including melanoma, carcinoma, and lymphoid types.

There was no relationship between the level of expression of the antigenic determinant recognized by MoAb 345.134S and other surface markers tested. The level of antigen detected by MoAb 345.134S was unchanged in the presence of interferon but was decreased by puromycin exposure. The opposite was true for cell-dependent lysis of cultured human cells. (32 Refs)

47. ALTERATIONS IN HEPATIC 5'-NUCLEOTIDASE IN THE TUMOR-BEARING RAT.

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Dept. Biochemistry and Microbiology, Univ. St. Andrews, Fife KY16 9AL, Scotland
Enzyme; 27(2):119-123 1982

The effect of the growth of the Walker 256 carcinoma on the level of 5'-nucleotidase and alkaline phosphatase in the whole liver and in an isolated hepatocyte membrane preparation of its host was investigated. Alkaline phosphatase activities of whole liver and plasma membrane were increased approx five-fold by tumor growth. A 50% decrease in whole liver 5'-nucleotidase activity was observed in tumor-bearing rats while the 5'-nucleotidase activity per milligram membrane protein was unaltered. Tumor growth would therefore appear to affect a pool of 5'-nucleotidase which is not associated with the plasma membrane. (Author abstract) (21 Refs)

48. IMMUNOLOGIC RECOGNITION OF CELL SURFACE ANTIGENS IN NORMAL MOUSE NEURAL TISSUES AND IN NEUROEPITHELIAL CELLS OF THE OTT-6050 MOUSE TERATOMA. A RADIOMETRIC, GEL ELECTROPHORETIC, AND MORPHOLOGIC (IMMUNOFLUORESCENCE AND IMMUNOPEROXIDASE) STUDY.

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Acta Neuropathol (Berl); 56(3):214-224 1982

Use of the immunoperoxidase method for recognition of neural cell surface antigens (SA) was studied. Membrane fractions were prepared from neural tissues, liver, spleen, kidney, heart, testes, and thymus of adult and neonatal F1(129/J x C3H/HeJ) mice and from three OTT-6050 mouse teratoma-derived tumors. Antiserum to neural SA was produced in New Zealand white rabbits by injection of membrane preparations. The antiserum cross-reacted with SA of neuroblasts and primitive and mature glial cells, as indicated by radioimmunoassays. Binding of the antiserum to neonatal brain membranes was almost completely eliminated by preabsorption on adult mouse brain membranes; binding was not reduced by preabsorption on liver, spleen, kidney, or heart membranes. Antiserum cross-reacted with certain non-neural tissue types including sperm and sperm-precursor cells, some epithelial cells in the medulla of adult mouse thymus, and various mesenchymal cells in neonatal mice. Binding of antiserum to brain membranes was slightly reduced by preabsorption with testis pellets or thymus cells. Cross-reactivity was eliminated by preabsorption with adult brain membranes. Preabsorption with testis pellets or thymus cells reduced staining of primitive neuroepithelial cells in teratoma-derived tumors; staining of neural cells in the mature neuropil was reduced but not eliminated. Polyacrylamide gel electrophoresis identified major protein bands at mol wt 26,000, 32,000, 40,000, 42,000, 45,000, and 54,000 daltons. Results of autoradiographic analysis indicated that the antiserum reacted only with minor protein components of mouse brain membranes. (38 Refs)

49. HUMAN THY-1: EXPRESSION ON THE CELL SURFACE OF NEURONAL AND GLIAL CELLS.

Kemshead JT, Ritter MA, Cotmore SF, Greaves MF
Membrane Immunology Lab., Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Brain Res; 236(2):451-461 1982

The expression of the cell surface glycoprotein Thy-1 in a variety of human neuronal and neuroglial cell lines and fresh neuronal tumor tissue was examined using immunofluorescent, radioimmune binding and quantitative absorption techniques. Amount and distribution of Thy-1 were examined. Human Thy-1 was identified with a rabbit anti-pure rat brain Thy-1 antiserum, and the extent to which its antigenic determinants are shared with rodent Thy-1 was analyzed. Thy-1 was present on the cell surfaces of 4 human neuronal lines, 2 glial cell lines, and most fresh neuroblastoma material (both primary tumor and bone marrow metastases), but with considerable heterogeneity of antigen expression. Binding of rabbit anti-rat brain Thy-1 antibodies was specific for the Thy-1 molecule: binding was completely blocked by prior absorption of the antibody with either human or rat pure Thy-1. Approx 25% f the total anti-rat Thy-1 antibodies in the reagent will bind to the human homolog of Thy-1. The Thy-1 antigen in humans seems to be associated with neuronal cells, as in rats and mice, although the antigen may be more common in the human nervous system. (27 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

50. TURNOVER OF MITOCHONDRIAL INNER MEMBRANE PROTEINS IN HEPATOMA MONOLAYER CULTURES.

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Dept. Biochemistry, Sch. Medicine, Oregon Health Sciences Univ., Portland, OR, 97201
J Biol Chem; 257(7):3575-3580 1982

The degradation rates of inner mitochondrial membrane proteins were examined in serum-deprived hepatoma cultures. In those nonproliferating cells, the degradation of composite mitochondrial proteins was a first order process with a half-life of 34 hr. The half-lives of specific inner mitochondrial membrane polypeptides were determined by examining the 3H/35S of isolated polypeptides from cells given [3H]methionine and [35S]methionine pulses, respectively, before and after a 2-day chase period. The 33 most abundant polypeptides resolved on a bidirectional polyacrylamide gel system showed half-lives ranging from 20 to 100+ hr. The 15 polypeptides translated on mitochondrial ribosomes in the presence of inhibitory concentrations of cycloheximide also displayed heterogeneous rates of degradation (t 1/2 = 35-100+ hr). None of the isolated adenosine triphosphatase (coupling factor F1) or immunoprecipitated cytochrome c oxidase subunits were significantly turned over during the chase period. Five of eight cytochrome b-c1 complex subunits, however, were turned over significantly more rapidly (t 1/2 = 39-42 hr) than the other three (t 1/2 = 94+ hr). The results demonstrate heterogenous degradation rates for inner membrane polypeptides, extending in some cases to those within the same respiratory complex. (Author abstract) (49 Refs)

51. THE BACTERIAL RIBOSOME: A PROGRAMMED ENZYME.

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Angew Chem [Engl]; 21(1):23-36 1982

An introduction to the steps involved in ribosome-dependent protein synthesis and to the structures of the compounds involved is presented. An attempt is made to elucidate the mechanistic principles of the ribosome as a programmed enzyme. Topics include ribosome-dependent protein biosynthesis; structure and function of the ribosomal machinery; transfer ribonucleic acids; messenger RNA, the program for the biosynthesis of proteins; proteins that function as enzymes, carriers, and modulators of transfer RNA conformation; selection of the beginning of a gene and framing the messenger RNA by formation of the initiation complex; and peptide chain elongation in protein biosynthesis. (89 Refs)

52. EFFECTS OF CADMIUM ON MOUSE LIVER POLYRIBOSOME FUNCTION.

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Exp Cell Biol; 50(2):72-78 1982

The distribution of ribosomes between their various functional states (native subunits, unprogrammed ribosomes, monoribosomes and polyribosomes) was analyzed in livers of cadmium-treated (CdCl2, 20 umol/kg, 1 hr) and control mice. The ribosomes with double-labeled RNA were separated by sucrose density-gradient centrifugation in zonal rotor, and monomeric ribosome fraction was subsequently isolated and analyzed by selective dissociation of unprogrammed ribosomes. The analysis shows that the increase in monomeric ribosomes, occurring during polyribosome disaggregation in livers of cadmium-treated mice, is entirely due to the increase of the unprogrammed ribosome fraction at the expense of polyribosomes. Protein synthetic activity of polyribosomes in vivo in livers of cadmium-treated and control mice were compared by double labeling of nascent and soluble polypeptides with [14C]- and [3H]-leucine. Incorporation of radioactivity was relatively higher in nascent polypeptides and lower in soluble polypeptides in cadmium-treated as compared with control mice. The results indicate that cadmium inhibits protein synthesis in livers of mice, affecting both the rate of initiation and the rate of elongation, but decreasing the former more than the latter. (Author abstract) (15 Refs)

53. HEMIN-INDEPENDENT CONTROL OF GLOBIN SYNTHESIS IN FRIEND ERYTHROLEUKEMIA CELLS INDUCED TO DIFFERENTIATE.

Stringer EA, Friend C
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Proc Natl Acad Sci USA; 79(6,part1):1839-1843 1982

A hemin-independent translational inhibitor that prevents synthesis of rabbit globin when uninduced Friend leukemia (FL) cell and rabbit reticulocyte lysates are mixed cannot be detected in FL cells induced to differentiate. Mixing of lysates of FL cells induced with hexamethylene bisacetamide or aminonucleoside of puromycin and rabbit reticulocytes does not cause inhibition of rabbit globin synthesis. Induction also results in the cells acquiring sensitivity to the inhibitor from uninduced FL cells. A reduction in total protein synthesis is observed when uninduced and induced FL cells lysates are mixed. Inhibition does not result from competition by an excess of uninduced FL cell mRNA species for the translational machinery because uninduced FL cell lysates retain their inhibitory activity after treatment with micrococcal nuclease. Rabbit globin mRNA recovered from rabbit reticulocyte lysates that have been incubated with lysates of uninduced FL cells can still be translated effectively, indicating that inhibition does not result from modification of other species of mRNA by uninduced FL cell lysates. A switch to hemin-dependent translational control does not follow induction of differentiation. The rate of amino acid incorporation in induced FL cell lysates, like that in uninduced FL cell lysates, is unaffected by omission of exogenous hemin from the system. Its presence is not required to prevent activation of heme-regulated inhibitor. From these data, it is clear that the control of protein synthesis in FL cells, whether or not they are induced, is different from that regulated by hemin in normal erythroid cells. (Author abstract) (32 Refs)

54. CONTROL OF IGM SYNTHESIS IN THE MURINE PRE-B CELL LINE, 70Z/3.

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Dept. Genetics (SK-50), Univ. Washington, Seattle, WA, 98195
J Immunol; 128(4):1664-1670 1982

The murine 70Z/3 tumor resembles a pre-B cell in synthesizing only intracellular chains and no detectable light chain. However, one kappa gene is already rearranged, and after overnight incubation with lipopolysaccharide (LPS), most of the cells are induced to synthesize light chain. The induced cells display IgM on their surface, but do not secrete IgM. Thus, 70Z/3 cells resemble cells poised at the pre-B cell/B lymphocyte border. We have examined synthesis and post-translational modification of mu-chains in uninduced and induced 70Z/3 cells. Isolation of mu-chains and peptide maps demonstrated that both populations synthesize intracellular forms that correspond to membrane-specific mum and secretion-specific mus chains. These intracellular forms have completed only the first of the two glycosylation steps characteristic of eukaryotic cells. After induction by LPS, L chain synthesis commences, mum and mus synthesis are both increased twofold to threefold (due to an increased rate of synthesis rather than decreased degradation), and both complex with L chain to form mu2L2 tetramers. Furthermore, the glycosylation of a subset of the mum chains is completed, and these are placed on the membrane. However, unglycosylated mu2L2 tetramers can be placed on the membrane, so glycosylation is not a requirement. These data suggest that L chain may not be sufficient for externalization of mum and mus chains. These data support the idea that the controls of membrane placement and secretion of mu chains are post-translational and that different mechanisms operate for mum and mus chains. (Author abstract) (61 Refs)

55. REGULATION OF THYMIDINE KINASE ENZYME LEVEL IN SERUM-STIMULATED MOUSE 3T6 FIBROBLASTS.

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Dept. Biochemistry, Ohio State Univ., Columbus, OH, 43210
Exp Cell Res; 138(1):79-85 1982

This report describes the regulation of thymidine kinase (TK) enzyme level in mouse 3T6 fibroblasts experiencing a serum-induced transition between the resting (G0) and growing states. The specific activity of TK is about 200-500 pmoles TMP formed x min-1 x mg protein-1 in exponentially growing cells but is scarcely detectable (usually less than 5 pmoles TMP formed x min-1 x mg protein(-1)) in cells resting at confluence in medium containing 0.5% serum. When the resting cells are serum-stimulated to re-enter the cell cycle, TK activity remains at the resting level until about 12 hr following stimulation, but then increases dramatically as the cells enter S phase. Experiments with inhibitors of protein or RNA synthesis indicate that the increase in enzyme level is the result of de novo synthesis of the enzyme, and that the increase depends on transcription. The increase in TK level is not blocked when cells are stimulated in the presence of inhibitors of DNA synthesis, indicating that there is no tight coupling between these two processes. The increase is not blocked by the presence of thymidine ranging in concentration from 10(-7) to 10(-3) M, suggesting that fluctuations in the intracellular concentrations of thymidine or thymidylic acid do not affect the increase in TK level. When cells are treated with cycloheximide, the level of TK decreases with a 4-5 hr half-life, indicating that the enzyme is relatively unstable. When the serum stimulus is withdrawn from cells in S phase (20 hr following stimulation), the TK level decreases with the same 4 hr half-life beginning about 6 hr following serum withdrawal. The ability of the cell to control the rate of production of TK over a very wide range, along with the relative instability of the enzyme appear to be responsible for the rapid and wide variations in TK level during the cell cycle. The increase in the level of TK appears to be regulated in a manner similar to that of dihydrofolate reductase and thymidylate synthetase in serum-stimulated 3T6 cells, suggesting that the expression of these genes (and perhaps those for other S phase enzymes) may be coordinated by a common control signal. (Author abstract) (23 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

56. EFFECT OF HEAT SHOCK ON GENE EXPRESSION IN HUMAN EPIDERMOID CARCINOMA CELLS (STRAIN KB) AND IN PRIMARY CULTURES OF MAMMALIAN AND AVIAN CELLS.

Atkinson BG, Pollock M
Cell Science Lab., Dept. Zoology, Univ. Western Ontario, London, Ontario N6A 5B7, Canada
Can J Biochem; 60(3):316-327 1982

The brief incubation of human epidermoid carcinoma (KB) cells, and of primary cultures of quail myoblasts and hamster fibroblasts, at an elevated temperature causes the pattern of gene expression to shift from the production of a broad spectrum of different proteins to the enhanced synthesis of a small number of heat-shock proteins. Comparison of the heat-shock polypeptides synthesized by each of these vertebrate cells demonstrates the similarity of some, as well as the uniqueness of other, heat-inducible gene products synthesized by cells from different vertebrates. A major polypeptide, commonly synthesized in response to heat by each of these vertebrate cells, has an apparent mol wt of 64,000 and an isoelectric point of 5.8. Triton X-100 completely extracts this polypeptide from quail myoblasts and hamster fibroblasts, and partially extracts it from KB cells. This particular response to heat shock, by cells from different vertebrates, suggests that it may involve the expression of a gene(s) with an analogous and potentially crucial cellular function. This specific heat-shock polypeptide, as well as others, is not detectably synthesized in quail cells prior to heat shock or 6-8 hr after recovery from heat shock which suggests that in this cell type it may be a product of a normally quiescent gene(s) and that its expression is subject to thermal regulation. (Author abstract) (45 Refs)

57. TUMOR PROMOTERS INCREASE THE SYNTHESIS OF A 32,000-DALTON PROTEIN IN BALB/C 3T3 CELLS.

Hiwasa T, Fujimura S, Sakiyama S
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Proc Natl Acad Sci USA; 79(6,part1):1800-1804 1982

The effect of 12-O-tetradecanoylphorbol 13-acetate (TPA), a potent tumor promoter, on the synthesis of proteins in BALB/c 3T3 mouse fibroblasts has been studied. When [35S]methionine-labeled proteins synthesized after the addition of TPA were analyzed by one- or two-dimensional polyacrylamide gel electrophoresis, the increased synthesis of a 32,000-dalton protein (designated p32) was noted as one of the earliest changes. The synthesis of p32 increased approx two-fold within 2 hr at a promoter concentration of 20 ng/ml. Phorbol 12,13-didecanoate, another potent tumor promoter, showed the same effect as TPA, whereas 4-O-methyl-TPA, which has little promoting activity, did not enhance the synthesis of p32 at the same concentration but did effect a slight increase at 1 ug/ml. p32 differed immunologically from a major excreted protein discovered by Gottesman. The increased rate of the synthesis of p32 was considered to be regulated at the transcriptional level, because the increase in synthesis of p32 was inhibited by actinomycin D, and TPA-treated cells were shown to contain a higher level of translatable mRNA coding for p32 than do control cells. A possible relationship between the increase in p32 synthesis and transformation is discussed. (Author abstract) (49 Refs)

58. DECREASED SYNTHESIS OF LARGE FUCOSYL GLYCOPEPTIDES DURING DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS INDUCED BY RETINOIC ACID AND DIBUTYRYL CYCLIC AMP.

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Dev Biol; 90(2):441-444 1982

The F9 clone of a teratocarcinoma stem cell line (EC) was induced to differentiate in vitro by retinoic acid (1 uM) and dibutyryl cyclic AMP (1 milliM). Synthesis of large fucosyl glycopeptides (belonging to the lactosaminoglycan series, with a galactose and N-acetylglucosamine core structure) declined as the F9 cells differentiated morphologically. The amount of fucose in the particulate fraction of cells (calculated from the protein content) declined slightly during cell differentiation. The differentiation system of F9 cells, with a clear-cut change in the high-mol wt carbohydrate profile during differentiation, is a suitable model for further study of the causal relationship between altered glycosylation and induction of cell differentiation. (14 Refs)

59. RADIATION-INDUCED ALTERATIONS IN SPLENIC ACID PHOSPHATASE OF PIGEONS.

Gadhia PK, Shah VC
Dept. Biosciences, South Gujurat Univ., Surat 395, 007 Gujarat, India
J Radiat Res (Tokyo); 22(4):443-448 1981

Biochemical and histochemical changes in acid phosphatase (AP) in the spleen of adult healthy pigeons (Columba livia intermedia Strickland) of both sexes (200-300 g) were studied after birds were subjected to a total body sublethal dose of gamma radiation (400 rad). Specific AP activity increased significantly 48 hr and 72 hr after irradiation. The enhancement of specific activities accompanied by a decline in percent 'bound' activities of the enzyme in the spleen indicate increased permeability of lysosomes, which may be due to structural modification in the lipoprotein membrane. The number of lymphocytes decreased drastically, but macrophages and reticular cells showed intense staining and increased AP activity. The increased AP activity in pigeon spleen is probably due to release of factor(s) by damaged lymphocytes (lymphocytolysis) after irradiation, which apparently induce an increase in the permeability of the lysosomal membrane. Pigeon spleen AP response to whole body radiation is similar to that previously reported for the rat. (15 Refs)

60. POSITIVE AND NEGATIVE CAMP-MEDIATED CONTROL OF TYROSINE AMINOTRANSFERASE SYNTHESIS IN REUBER H35 HEPATOMA CELLS.

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Laboratorium voor Moleculaire Celbiologie der Rijksuniversiteit te, Utrecht, Transitorium 3 Universiteits Centrum 'De Uithof', Padualaan 8, NL-3584-CH Utrecht, The Netherlands
Eur J Biochem; 123(1):217-222 1982

Induction of L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5) by 6,O-dibutyryl-adenosine 3',5'-monophosphate (Bt2cAMP) in Reuber H35 hepatoma cells reaches a maximum value between 3-5 hr after addition of Bt2cAMP and subsequently decreases in the continuous presence of BtcAMP. We have investigated the kinetics of the increase, ie, induction, and the decrease, ie the repressed state, of the tyrosine-aminotransferase-synthesizing system under these conditions. Our experimental results are as follows. (1) The repressed state of the tyrosine-aminotransferase-synthesizing system is not caused by a decrease in the intracellular cAMP concentration. (2) The repressed state is inhibited by actinomycin D (while induction is not inhibited). (3) During the repressed state no effect of dexamethasone on tyrosine aminotransferase synthesis is found, while during induction Bt2cAMP and dexamethasone act synergistically. (4) Longer starvation of the cells in serum-free medium has no influence on the kinetics of the induction/repressed state curve. From these results we have concluded that the mechanism of the transition to the repressed state of the tyrosine-aminotransferase-synthesizing system is essentially different from the mechanism of deinduction which occurs after removal of the inducer. Moreover, the repressed state of the system is a phenomenon which is induced by Bt2cAMP separately from induction at a different level of protein synthesis. (Author abstract) (39 Refs)

DIETARY AND NUTRITIONAL EFFECTS

61. CELL-MEDIATED IMMUNITY IN MALNOURISHED GUINEA PIGS AFTER MYCOBACTERIUM BOVIS BCG VACCINATION.

McMurray DN, Yetley EA
Dept. Medical Microbiology, Texas A and M Univ., College Station, TX, 77843
Infect Immun; 35(3):909-914 1982

Specific-pathogen-free guinea pigs were vaccinated with viable Mycobacterium bovis BCG and maintained on purified, isocaloric diets containing either 30% or 7.5% casein, or commercial chow. At intervals of 4, 5, 6, and 8 weeks postvaccination, groups of guinea pigs from each diet treatment were skin tested with purified protein derivative and killed. Protein-deficient animals exhibited progressive reductions in total serum proteins and albumin. Significantly greater numbers of viable M bovis BCG were recovered from the vaccination site and inguinal lymph nodes of protein-deficient guinea pigs at all intervals. In contrast, the development of delayed hypersensitivity was markedly retarded in the 7.5% casein group and was also reduced somewhat in the 30% casein group as compared to chow control. Peripheral blood lymphocytes from protein-deficient animals did not respond normally in vitro to a polyclonal T cell mitogen, phytohemagglutinin. These results demonstrate that protein-calorie malnutrition in this model impairs the development of cell-mediated immunity as evidenced by skin test anergy, lymphocyte hyporesponsiveness, and failure to control levels of viable M bovis BCG after vaccination. (Author abstract) (34 Refs)

62. INFLUENCE OF METHIONINE ADMINISTRATION ON SERUM ALPHA-FETOPROTEIN LEVELS IN ETHIONINE-INJURED RATS.

Miyazaki M, Watanabe A, Wahid S, Tsunashima M, Sato J
Div. Pathology, Cancer Inst., Okayama 700, Japan
Res Exp Med (Berl); 180(1):15-19 1982

A single injection of ethionine (100 mg/100 g body wt) caused serum alpha-fetoprotein (AFP) levels to sharply increase in female adult Sprague 2 days later, reaching a max at 4 days, and then decreasing. After ingesting a diet containing 1% ethionine for 3 wk serum AFP constantly increased to a max level of 800 nanog/ml. Supplementing the ethionine diet with 2.8% methionine resulted in slightly elevated AFP on day 4, but no further elevation. (17 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

63. ORNITHINE DECARBOXYLASE: ESSENTIAL IN PROLIFERATION BUT NOT DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS.

Luk GD, Civin CI, Weissman RM, Baylin SB
Oncology Center, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
Science; 216(4541):75-77 1982

The ornithine decarboxylase inhibitor DL-alpha-difluoromethyl ornithine inhibited a proliferation-associated increase in ornithine decarboxylase activity in cultured human promyelocytic leukemia cells, resulting in a marked suppression of cell proliferation and subsequent cell loss. It also inhibited increases in ornithine decarboxylase activity associated with the phorbol ester-induced conversion of promyelocytic HL-60 cells to monocyte-like cells and the retinoic acid-induced conversion to granulocyte-like cells. However, the inhibition of ornithine decarboxylase activity did not prevent cellular differentiation. These results suggest that polyamine biosynthesis has a specific role in cell proliferation rather than in inducing differentiation that is not accompanied by proliferation. The data also demonstrate that cessation of proliferation in HL-60 cells is not necessarily associated with differentiation. (Author abstract) (18 Refs)

OTHER RELATED STUDIES

64. METHIONINE-ENKEPHALIN AND LEUCINE-ENKEPHALIN IN HUMAN SYMPATHOADRENAL SYSTEM AND PHEOCHROMOCYTOMA.

Yoshimasa T, Nakao K, Ohtsuki H, Li S, Imura H
Second Div., Dept. Medicine, Kyoto Univ. Sch. Medicine, Kyoto 606, Japan
J Clin Invest; 69(3):643-650 1982

To elucidate the physiological and pathophysiological significance of methionine- and leucine-enkephalin (Met- and Leu-enkephalin, respectively) in human sympathoadrenal system, the contents of these peptides in normal human sympathetic nervous system, adrenal medulla, and pheochromocytomas were determined by specific radioimmunoassays combined with reverse-phase high-performance liquid chromatography. Met-enkephalin-like and Leuenkephalin-like immunoreactivity (Met-enkephalin-LI and Leu-enkephalin-Li, respectively) were detected by radioimmunoassay in adrenal glands, adrenal medulla, stellate ganglia, sympathetic trunks, and celiac ganglia, and their contents in adrenal medulla were highest. Existence of authentic Met- and Leu-enkephalin was confirmed by reverse-phase high-performance liquid chromatography. Met-enkephalin was approx 74% of Met-enkephalin-LI, whereas Leu-enkephalin was approx 30% of Leu-enkephalin-LI in human adrenal medulla. The ratio of Met- to Leu-enkephalin was 2.6 in human adrenal medulla, whereas it was higher in sympathetic ganglia or trunks. In four cases of pheochromocytoma marked difference in Met- and Leu-enkephalin contents was found between medullary and extramedullary tumors. The contents were about three orders higher and the Met- to Leu-enkephalin ratio was lower in medullary than in extramedullary pheochromocytomas, reflecting the tissues where the tumors arose. These results suggest the physiological roles of Met- and Leu-enkephalin in sympathetic nervous systems and adrenal glands and their pathophysiological significances in pheochromocytomas. (Author abstract) (21 Refs)

65. MUTANT OF CHINESE HAMSTER OVARY CELLS WITH ALTERED MANNOSE 6-PHOSPHATE RECEPTOR ACTIVITY IS UNABLE TO SYNTHESIZE MANNOSYLPHOSPHORYLDOLICHOL.

Stoll J, Robbins AR, Krag SS
Dept. Biochemistry, Johns Hopkins Univ., Sch. Hygiene and Public Health, Baltimore, MD, 21205
Proc Natl Acad Sci USA; 79(7):2296-2300 1982

B4-2-1 is a Chinese hamster ovary cell mutant previously isolated and characterized as deficient in mannose 6-phosphate receptor activity. We show here that B4-2-1 is a pleiotropic mutant, defective in biosynthesis of asparagine-linked oligosaccharides. B4-2-1 is unable to synthesize mannosylphosphoryldolichol; the consequences of this defect on glycosylation are (i) biosynthesis of one major lipid-linked oligosaccharide, characterized by its resistance to endoglycosidase H and decreased size; this oligosaccharide is similar to a minor species of lipid-linked oligosaccharide found in parental cells; (ii) transfer of this oligosaccharide to newly synthesized proteins; and (iii) absence of normal 'high-mannose' oligosaccharides on mature glycoproteins isolated from B4-2-1; glycoproteins from the mutant contain complex oligosaccharides as well as endoglycosidase H-resistant, alpha-mannosidase-sensitive species. While the glycosylation defect may alter adversely the function of several glycoproteins in the mutant, including that of the mannose 6-phosphate receptor, it appears to have no effect on the formation or function of the mannose 6-phosphate recognition marker on acid hydrolases of B4-2-1. (Author abstract) (30 Refs)

66. MONOCLONAL ANTIBODIES THAT INHIBIT ENZYME ACTIVITY OF 3-METHYLCHOLANTHRENE-INDUCED CYTOCHROME P-450.

Park SS, Fujino T, West D, Guengerich FP, Gelboin HV
(c/o Gelboin) Lab. Molecular Carcinogenesis, NCI, NIH, Bethesda, MD, 20205
Cancer Res; 42(5):1798-1808 1982

Somatic cell hybrids were made between mouse myeloma cells and spleen cells derived from BALB/c mice immunized with liver microsomal cytochrome P-450 purified from rats treated with 3-methylcholanthrene (MC-P-450). Thirty-seven independent hybrid clones among 66 tested produced monoclonal antibodies to the MC-P-450 as measured by radioimmunoassay. More than 10 of the monoclonal antibodies formed were positive for MC-P-450 with respect to protein binding measured by radioimmunoassay, precipitation of the enzyme caused by antibody binding and enzyme aggregation, and inhibition of enzymatic activity. Analysis by gel electrophoresis indicated that a single microsomal protein band interacted with the antibody and that this band comigrated with MC-P-450. These monoclonal antibodies interacted with the major form of cytochrome P-450 from beta-naphthoflavone-induced rats as well as with MC-P-450 but did not bind, precipitate, or inhibit the activity of the major form of cytochrome P-450 from phenobarbital-treated rats. The monoclonal antibodies inhibited 7-ethoxycoumarin deethylase and benzo(a)pyrene hydroxylation activity of the purified MC-P-450 with varying degrees. Analysis of benzo(a)pyrene metabolism indicated that the monoclonal antibodies inhibited the enzyme activity of the purified MC-P-450 at all of the positions at which oxidation occurs. Analysis of antibody inhibition of benzo(a)pyrene metabolism by microsomes from 3-methylcholanthrene-treated rats showed an inhibition of phenol and diol formation that ranged from 46 to 72%. 1,6-Quinone production was not affected, which suggests that this metabolite was formed by another cytochrome P-450 isozyme or nonenzymatically. The purity, specificity, and potential immortality of their hybridoma cell source will make the monoclonal antibodies extraordinarily useful for the study of substrate and inducer specificity and in the identification and quantitative assay of multiple forms of the cytochrome P-450 and the determination of their content and function in different tissues, species and individuals. (43 Refs)

67. HYPERVISCOSITY SYNDROME IN PARAPROTEINEMIA MANAGED BY PLASMA EXCHANGE; MONITORED BY SERUM TESTS.

Beck JR, Quinn BM, Meier FA, Rawnsley HM
171 Harrison Ave., Boston, MA, 02111
Transfusion; 22(1):51-53 1982

Clinical and laboratory indices of hyperviscosity were studied in three patients presenting with this complication of paraproteinemia. Serum viscosity correctly predicted the presence of clinical hyperviscosity syndrome in patients with stable hemoglobin and albumin concentrations. Serum viscosity also correlated with total protein and paraprotein levels. A simple exponential model proved not only to describe the kinetics of blood viscosity during plasma exchange but also to predict protein concentration at symptomatic recovery. Construction of such a protein-viscosity curve helps manage by plasma exchange patients with paraproteinemia who develop hyperviscosity syndrome. (Author abstract) (9 Refs)

68. MOLECULAR CLONING OF A CDNA SEQUENCE ENCODING A TROPHECTODERM-SPECIFIC MARKER DURING MOUSE BLASTOCYST FORMATION.

Brulet P, Jacob F
Service de Genetique Cellulaire, College de France et de l'Institut Pasteur, 25 rue du Dr. Roux, 75015 Paris, France
Proc Natl Acad Sci USA; 79(7):2328-2332 1982

A cDNA clone has been isolated from a trophoblastoma cDNA library. The mRNA complementary to this sequence directs the in vitro synthesis of proteins. The two-dimensional electrophoretic pattern of migration of these proteins is superposable to that of the trophoblastoma intermediate filament proteins recognized by monoclonal antibody (mAb) TROMA-1. This mAb had previously been shown to label trophectoderm cells but not inner cell mass cells. With a sensitive binding assay (ultrasensitive enzymatic radioimmunoassay), these in vitro synthesized proteins were recognized by mAb TROMA-1. The proteins are immunoprecipitated by an antiserum directed against trophoblastoma intermediate filament proteins and by a serum directed against a major cytoskeletal protein found in murine extraembryonic endodermal cell lines (Endo-A). The cDNA sequence detects specific mRNA(s) migrating with 18S ribosomal RNA in trophoblastoma but not in embryonal carcinoma cells. (Author abstract) (19 Refs)

69. DETERMINATION OF THE BINDING OF 2,4,5,2',4',5'-HEXACHLOROBIPHENYL BY LOW DENSITY LIPOPROTEIN AND BOVINE SERUM ALBUMIN.

Becker MM, Gamble W
(c/o Gamble) Dept. Biochemistry and Biophysics, Oregon State Univ., Corvallis, OR, 97331
J Toxicol Environ Health; 9(2):225-234 1982

A method of measuring protein binding of polychlorinated biphenyls (PCB) that reduces error due to PCB binding to filtering apparatus is described and applied to the analysis of binding of 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) to bovine serum albumin (BSA) and low density lipoprotein (LDL). LDL bound HCB at several noninteractive sites. BSA bound HCB at one noninteractive site. The assay technique employs toluene to wash the cellular triacetate membrane filter. Results showed that 91.2% of HCB appears in the first toluene extraction and 8.2% in subsequent extractions. BSA and LDL bind HCB effectively, suggesting that plasma proteins may have a role in HCB distribution to peripheral cells. (23 Refs)

70. BIOSYNTHESIS OF CARNOSINE AND RELATED PEPTIDES BY GLIAL CELLS IN PRIMARY CULTURE.

Bauer K, Hallermayer K, Salnikow J, Kleinkauf H, Hamprecht B
Institut fur Biochemie and Molekulare Biologie, Technische Universitat Berlin, Franklinstrasse 29, D-1000 Berlin, W. Germany
J Biol Chem; 257(7):3593-3597 1982

Synthesis of carnosine (beta-alanylhistidine) and related peptides by glial cells in primary culture could be demonstrated. After incubation with [3H]beta-alanine, the radiolabeled dipeptides could be isolated from the cell extract and the culture medium. With gamma-amino[3H]butyric acid, however, rapid degradation of the tracer without significant synthesis of homocarnosine (gamma-aminobutyrylhistidine) was observed. Aminooxyacetic acid, a known inhibitor of gamma-aminobutyryl:alpha-ketoglutarate aminotransferase, inhibits the degradation of gamma-amino[3H]butyric acid very strongly and of [3H]beta-alanine partially. After preincubation of the cells with this inhibitor, incorporation of gamma-amino[3H]butyric acid into homocarnosine and related peptides could be demonstrated. (Author abstract) (29 Refs)

71. UNIQUE CHARACTERISTICS OF RAT SPLEEN LYMPHOCYTE, L1210 LYMPHOMA AND HELA CELLS IN GLUTATHIONE BIOSYNTHESIS FROM SULFUR-CONTAINING AMINO ACIDS.

Brodie AE, Potter J, Reed DJ
Dept. Biochemistry and Biophysics, Oregon State Univ., Corvallis, OR, 97331
Eur J Biochem; 123(1):159-164 1982

Suspensions of rat spleen lymphocyte, murine L1210 lymphoma and HeLa cells were partially depleted of glutathione (GSH) with diethyl maleate and allowed to utilize either 35S methionine, 35S cystine or 35S cysteine for GSH synthesis. Lymphocytes preferentially utilized cysteine, compared to cystine, at a ratio of about 30 to 1, which was not related to differences in the extent of amino acid uptake. Only HeLa cells displayed a slight utilization of methionine via the cystathionine pathway for cysteine and GSH biosynthesis. HeLa and L1210 cells readily utilized either cystine or cysteine for GSH synthesis. The three cell types accumulated detectable levels of intracellular cysteine glutathione mixed disulfide when incubated in a medium containing a high concentration of cystine. Various enzyme activities were measured including gamma-glutamyl transpeptidase, GSH S-transferase and gamma-cystathionase. These results support the concept of a dynamic interorgan relationship of GSH to plasma cyst(e)ine that may have importance for growth of various cell types in vivo. (Author abstract) (30 Refs)

72. REGULATION OF AMINO ACID TRANSPORT INTO EHRLICH CELLS. EFFECT OF 3',5'-ADENOSINE MONOPHOSPHATE ON L-LEUCINE TRANSPORT.

Cuesta Casado MC, Ramos EH
Instituto de Quimica Biologica, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Argentina
Cell Mol Biol; 27(6):635-641 1981

The effect of adenosine 3',5'-cyclic monophosphate on L-leucine transport was studied regarding its regulatory action on one parameter under pleiotypic control. The metabolic processes related to biosynthesis and degradation of cAMP operate in Ehrlich cells. An increase in the intracellular content of the cyclic nucleotide partially inhibits the transport of L-leucine. The regulatory effect is maintained in all stages of tumor growth. In vitro experiments showed no effect of cAMP or Bu2cAMP on (Na+ - K+)-ATP-A activity of plasma membranes under the same experimental conditions as in in vivo experiments. (Author abstract) (20 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Adachi T 26
Akahonai Y 45
Alitalo K 29
Atkinson BG 56
August JT 43
Avner P 25
Babiarz B 37
Baker MA 13
Bakka A 8
Bannikov GA 14
Barrach HJ 18
Bastin JM 36
Bauer K 70
Baugher BW 7
Baylin SB 63
Beck JR 67
Becker MM 69
Belvedere G 6
Blanchard SG 34
Blezza D 6
Blumberga IA 1
Bolarin DM 19
Borrello S 3
Borrello S 4
Bracaglia R 4
Bradley TR 27
Breton-Gorius J 36
Bricker MD 33
Brodie AE 71
Brulet P 68
Buck CA 39
Cantoni L 6
Carnevali P 4
Carter WG 38
Chang KJ 34
Civin CI 63
Clark CM 47
Coleman PS 35
Conklyn M 12
Cooke BA 32
Cooper SM 33
Cotmore SF 49
Cuesta Casado M 72
Daddona PE 7
Damsky CH 39
De Villez RL 41
Deschamps JF 36
Dix CJ 32
Dron' LV 1
Dupont B 35
Eckel RW 34
Edde B 9
Endresen L 8
Eng LF 48
Engel J 22
Feizi T 30
Ferrone S 46
Fijiyama J 44
Forgue-Lafitte 31
Friend C 53
Fujimura S 57
Fujino T 66
Furthmayr H 22
Furukawa T 44
Gadhia PK 59
Gamble W 69
Gamulin S 52
Gassen HG 51
Gelboin HV 66
Glebov AV 5
Goodlad GA 47
Greaves MF 49
Gros F 9
Guengerich FP 66
Guichard J 36
Hallermayer K 70
Hamprecht B 70
Hare JF 50
Hascall VC 18
Herman MM 48
Higashida H 16
Hiwasa T 57
Hodges R 50
Hodgson GS 27
Hoessli DC 11
Hughes EN 43
Iavorkovskii LI 1
Imai K 45
Imai K 46
Imura H 64
Ishizeki J 15
Jacob F 68
Jeantet C 9
Johnsen AS 8
Johnson LF 55
Kano-Tanaka K 16
Kao RT 24
Karran P 17
Kato T 16
Kawaharada M 45
Kay NE 46
Kelley WN 7
Kempf J 2
Kemshead JT 49
Kimata K 18
Kimura JH 18
Kirsch E 21
Kivirikko KI 19
Kleinkauf H 70
Knudsen KA 39
Krag SS 65
Krieg T 21
Kriegler AB 27
Kurkinen M 29
Laburthe M 31
Landi FL 3
Landi FL 4
Lane BC 33
Lewandrowski K 40
Li S 64
Liebes LF 12
Luk GD 63
Mains PE 54
Mattes PM 7
McCormick PJ 37
McMichael AJ 36
McMurray DN 61
McNiece IK 27
Meier FA 67
Mellstrom K 29
Midorikawa O 26
Millis AJ 37
Miyazaki M 62
Motoyama T 20
Muench AJ 55
Muller PK 21
Muramatsu H 25
Muramatsu H 58
Muramatsu T 25
Muramatsu T 44
Muramatsu T 58
Nagai Y 20
Nagatsu T 16
Nakano T 16
Nakao K 64
Nakazato Y 15
Narancsik P 52
Natali PG 46
Nevria D 12
Noguchi T 44
Odermatt E 22
Ohtsuki H 64
Oliver JM 23
Oshima RG 10
Ott U 22
Ozawa M 44
Palka JA 28
Park SS 66
Pawlicka E 28
Poddubnaia EV 1
Pollock M 56
Portier MM 9
Potter J 71
Quinn BM 67
Quirin-Stricker 2
Ramos EH 72
Ramsay PB 48
Rao LG 55
Rawnsley HM 67
Reed DJ 71
Remold-O'Donnel 40
Rennard SI 18
Ritter MA 49
Robbins AR 65
Rosselin G 31
Rothfield NF 23
Rubinstein LJ 48
Rugstad HE 8
Rungger-Brandle 11
Sahuquillo C 9
Sakakibara K 20
Sakiyama S 57
Salnikow J 70
Sato J 62
Sato K 44
Savolainen ER 19
Schmitt M 2
Schumacher M 32

Sedgwick B 17
Seits IF 5
Senecal JL 23
Serri F 3
Serri F 4
Shah VC 59
Sibley CH 54
Silber R 12
Snoek GT 60
Stark R 12
Stasko T 41
Stern R 24
Stevens S 17
Stoll J 65
Stringer EA 53
Suzuki T 20
Takahashi K 15
Thonar EJ 18
Timpl R 21
Timpl R 22
Titeux M 36
Troianovskii SM 14
Tsunashima M 62
Vaheri A 29
Vainchenker W 36
Van Rymenant ME 42
Van Wijk R 60
Van der Hofstad 42
Vandenberg SR 48
Virtanen I 29
Voorma HO 60
Wada T 45
Wahid S 62
Watanabe A 62
Watanabe H 20
Weber L 21
Weissman RM 63
West D 66
Wiche G 13
Wilson BS 46
Wilson JM 7
Wong M 24
Yachi A 45
Yamaguchi H 15
Yang SY 35
Yetley EA 61
Yoshimasa T 64
Zweibaum A 31

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In this issue:

- **NUCLEOPROTEINS** **1-6**
- **CYTOPLASMIC AND CONTRACTILE PROTEINS** **7-19**
- **PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS** **20-36**
- **BINDING/ RECEPTOR PROTEINS** **37-48**
- **MEMBRANE/ SURFACE PROTEINS** **49-60**
- **STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS** **61-67**
- **EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM** **68-76**
- **DIETARY AND NUTRITIONAL EFFECTS** **77-79**
- **POLYAMINES - SYNTHESIS AND METABOLISM** **80-85**
- **OTHER RELATED STUDIES** **86-102**

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NUCLEOPROTEINS

1. THE H1 HISTONES AND THEIR INTERPHASE PHOSPHORYLATED STATES IN DIFFERENTIATED AND UNDIFFERENTIATED CELL LINES DERIVED FROM MURINE TERATOCARCINOMAS.

Lennox RW, Oshima RG, Cohen LH
Inst. for Cancer Res., Fox Chase Cancer Center, Philadelphia, PA, 19111
J Biol Chem; 257(9):5183-5189 1982

The histone H1 fraction from the murine embryonal carcinoma cell line F9 can be resolved by two-dimensional gel electrophoresis into at least 15 components. Ten of these components incorporate 32P from administered orthophosphate. The digestion of 3H- and 32P-labeled samples with alkaline phosphatase indicates that this cell has at least five H1 subtypes, three of which have not been observed in the mouse before. Every subtype is phosphorylated to some extent. There are, however, striking differences among them in the degree of phosphorylation in interphase, both in the proportion of molecules present as phosphorylated forms and in the numbers of phosphate groups per molecule. Phosphorylations of two types are discernible; most phosphorylations do not significantly affect the mobility of the protein in sodium dodecyl sulfate-containing gels but one does, probably reflecting a phosphorylation-induced alteration in protein conformation. Four subtypes undergo only the first type of phosphorylation; the other subtype undergoes both types of phosphorylation. The same 5 subtypes are also present in two other independently derived embryonal carcinoma cell lines. In contrast, two differentiated (endoderm-like) cell lines derived from teratocarcinomas lack H1b, and one also lacks H1d. Our results are consistent with the view that the H1 subtypes differ from each other functionally. They also show that neither H1b nor H1d is essential for cell division. (Author abstract) (59 Refs)

2. PURIFICATION AND CHARACTERIZATION OF A NOVEL DNA EXONUCLEASE FROM MOUSE MYELOMA (MEETING ABSTRACT)

Becerra SP, Wilson SH
National Institutes of Health, Bethesda, MD, 20205
Fed Proc; 41(3):1195 1982

Preparations of helix destabilizing protein-1 from mouse myeloma contain a novel DNA exonuclease. This enzyme can be separated from the helix destabilizing protein and obtained in highly purified form. A 41,000-Mr polypeptide is a main constituent of the purified enzyme and comigrates with the exonuclease activity during the final step of the purification, Sephacryl S-200 gel filtration where the enzyme has a native Mr of 40,000. Overall purification of the enzyme activity is approximately 20,000-fold. This enzyme releases 5' oligonucleotides from ssDNA substrates, requires Mg++ for activity and does not degrade dsDNA or RNA. With 3' end labeled (pdA)55 as substrate, greater than 95% of the labeled products are (pdA)4 and revealed by electrophoresis in 23% polyacrylamide, 7 M urea gels; with 5' end labeled (pdA)55 as substrate, the main labeled product is (pdA)2, but some release of (pdA)3, (pdA)4, (pdA)5, and (pdA)6 occurs also. The rate of product release from these 3' end and 5' end labeled substrates is nearly identical at 37 C. The size distribution of products is independent of the percentage of substrate consumed. With uniformly labeled (pdA)55 as substrate, the size distribution of labeled products is almost, but not exactly, the same as with the 5' end labeled substrate. (no Refs)

3. HUMAN LIVER NUCLEOLAR ANTIGENS (MEETING ABSTRACT)

Busch RK, Yeoman LC, Spohn WH, Busch H
Department of Pharmacology, Baylor College of Medicine, Houston, TX, 77030
Proc Am Assoc Cancer Res; 23:1072 1982

A basic and acidic antigen were identified in liver extracts by rocket immunoelectrophoresis. To gain more information about the liver nuclear and nucleolar antigens, affinity chromatography with liver nucleolar antibodies coupled to Sepharose 4B was used to purify antigens from human liver nuclear Tris-HCl extracts. One antigen with MW 67,000 and pI 6.3 was found in liver but not HeLa nuclear Tris-HCl extracts. To identify other human liver nuclear and nucleolar antigens, the 'Western' blotting method was used, two liver antigens with MW 38,000 and 20,000 were identified. Another protein with MW 55,000 and pI 7.2 was abundant in liver NaCl-EDTA extracts but was found in much lower amounts, if at all, in human pheochromocytoma and HeLa cells. It was less abundant in cytosol and nucleoli of liver. Protein 55/7.2 was purified to electrophoretic homogenity; amino acid composition showed it contained 12.9% Gly, 11.4% Glu and 8.2% Asp. The A/B amino acid ratio of protein 52/7.2 was 1.5 and it had a notably high Alu/Leu ratio of 0.8 compared to the usual 0.5 ratio. (1 Ref)

4. GENERAL FEATURES OF ORGANIZATION OF TUMOR CELL NUCLEI.

Zbarskii IB
N. K. Kol'tsov Inst. Biology Development, Moscow, USSR
Vestn Akad Med Nauk SSSR; (3):3-10 1982

Literature data on the morphological, physiopathological, and biochemical properties of tumor cells are reviewed with special emphasis on the tumor-specific properties of the cell nucleus. Characteristic biochemical properties of tumor cells include decrease of superoximutase and glutathione-peroxidase activities, elevated cyanide-resistant formation of superoxide radicals in nuclear membranes, and increase of the content of alkali-insoluble residual fraction. Fractionation of nuclear matrix from normal liver and Zajdela hepatoma showed the predominance of high mol wt proteins in all fractions of Zajdela hepatoma, compared with exclusive localization of the high mol wt protein from the normal liver in the third fraction. Similar predominance of high mol wt proteins was observed in the nuclear matrix of hepatoma 27 cells. Indirect immunofluorescence showed that the residual protein of hepatoma 27 contained specific antigen, which was absent in the nuclei of normal liver cells. (62 Refs) (In RUS)

5. DIFFERENT HISTONE FAMILIES IN INTRACELLULAR SV40 NUCLEOPROTEIN COMPLEXES WITH RESPECT TO THE ACETYLATION TURNOVER.

Vesco C, Fantuzzi LF
Institute Cell Biology CNR, Via Romagnosi 18A, 00196 Roma, Italy
Virology; 118(2):389-400 1982

The turnover of normal and butyrate-induced acetylation in the histones complexed with intracellular SV40 DNA was analyzed in infected CV-1 cells. The analysis concerned the two principal structures containing the intracellular SV40 chromatin: the 75 S complexes and the provirions. It was found that, while the normal acetylation in the 75 S chromatin turns over at a lower rate than that of the host-cell chromatin, the hyperacetylation induced by butyrate turns over at a rate very close to that of cell chromatin. This distinguishes two families of 75 S histones, presumably corresponding to nucleosomes with different accessibility to histone-modifying enzymes. A third type of turnover is shown by the provirion histones, in which the normal acetylation occurs at a low rate but is highly conserved. The response to butyrate in these structures is either absent or similar to that of the 75 S histones. In the chromatin of the late SV40 mutant tsB11, histone hyperacetylation does not occur when infection proceeds at the restrictive temperature for the coat-protein assembly. The hyperacetylation of histones which characterizes the morphogenesis of SV40 appears to be an assembly-dependent process, starting in the 75 S chromatin and building up mainly in the provirions. (Author abstract) (38 Refs)

6. ISOLATION AND PARTIAL CHARACTERIZATION OF A NUCLEAR ANTIGEN (68/6.3) FROM THE NAMALWA CELL LINE (A BURKITT LYMPHOMA).

Chan PK, Frakes RL, Busch RK, Busch H
(c/o H. Busch) Dept. Pharmacology, Baylor Coll. Medicine, Houston, TX, 77030
J Cancer Res Clin Oncol; 103(1):7-16 1982

A nuclear antigen was purified from the 0.01 M Tris-HCl/pH 8 extract of nuclei of the Burkitt tumor Namalwa cell line to electrophoretic homogeneity by DEAE cellulose chromatography, affinity chromatography, and preparative isoelectric focusing. The yield of antigens was 0.02% of the nuclear 0.01 M Tris-HCl/pH 8 extract. On two-dimensional gel electrophoresis, the major antigen separated into two adjacent protein spots with mol wt of 68,000 and an approx pI of 6.3 (68/6.3 A and 68/6.3 B). A minor antigen had a mol wt of 61,000 and pI of 6.0 (61/6.0). Fourteen 125I-labeled peptides were obtained from the tryptic digest of the major antigen (68/6.3 A and 68/6.3 B). The amino-acid composition analysis of the purified antigens indicated that the amino acids in the highest content were glycine (15%), glutamic acid (11.6%), and serine (9%); the ratio of acidic to basic amino acids were 1.95. In studies on nucleolytic activity, the purified antigen produced a single-stranded and then a double-stranded cleavage of PM 2 and pBR 322 DNA. This antigen is the first purified nuclear antigen that reacts with the HeLa-specific nucleolar antibodies. (Author abstract) (28 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

7. DOMAIN STRUCTURE OF TERMINAL TRANSFERASE (MEETING ABSTRACT)

Riley LK, Coleman MS
Department of Biochemistry, University of Kentucky, Lexington, KY, 40536
Fed Proc; 41(3):1197 1982

Terminal deoxynucleotidyl transferase (TdT) from thymus tissue and human leukemic cells has an unusual protein structure which consists of protease-resistant domains linked by sensitive hinge regions which can be readily cleaved by proteases present within the tissues. All of the native forms of TdT isolated exhibit full catalytic activity. Identification of cleavage products of TdT can be specifically detected following sodium docecyl sulfate polyacrylamide gel electrophoresis of crude preparations and electrophoretic transfer of proteins to cellulose nitrate. Protein bands with TdT antigenic determinants are indicated by sequential incubation with anti-TdT IgG followed by anti-IgG conjugated with peroxidase. In extracts from all TdT containing tissues (calf and human thymus and human lymphoblasts), the protein exists as a single high molecular weight species of Mr 62K. Upon freezing, TdT human thymus is susceptible to proteolysis and appears as Mr 15K. Human leukemic marrow, unlike peripheral lymphoblasts, contains TdT molecular weight variants of Mr 62K, 58K, 45K, and beta subunit (Mr 31K), but no alpha subunit (Mr 12K). Purification of thymic TdT with protease inhibitors results in step-wise degradation to alpha and beta subunits. The alpha subunit is 20 times more antigenic than the beta subunit. In all tissues TdT appears to be synthesized at high molecular weight which can cleaved to smaller forms. (no Refs)

8. ACTIN STATE BEFORE AND AFTER LECTIN STIMULATION IN NORMAL LYMPHOCYTES AND NEOPLASTIC CELL LINES (MEETING ABSTRACT)

Rao KM, Varant J
The University of Michigan, Ann Arbor, MI, 48109
Fed Proc; 41(3):739 1982

The amount of monomeric (G-actin), polymeric (F-actin) and total actin in various cell types was determined using DNase I inhibition assay. Normal peripheral blood lymphocytes had approximately 70% of the actin in monomeric form. On stimulation with concanavalin A (con A), a con A concentration dependent decrease in the amount of monomeric actin was observed. At the optimal concentration of the mitogen (10 ug/ml) only 30% of the actin was found to be monomeric. The change in actin state could be inhibited by pretreatment with cytochalasin B. These observations indicate that stimulation of lymphocytes with con A leads to actin polymerization. In contrast, CEM cells, a T-cell derived lymphoblastoid cell line, had approximately 67% of the actin in polymeric state to begin with and treatment with con A resulted in no significant change. Similarly, K562, a myeloid cell line had about 70% of the actin in polymeric state and demonstrated no significant response to con A treatment. These observations suggest that the state of actin within the cell might play an important role in its ability to respond to external stimuli. Further, it is a possibility that the cell growth may be regulated by the degree of actin polymerization. (no Refs)

9. ACTIN-BINDING PROTEINS - REGULATORS OF CELL ARCHITECTURE AND MOTILITY.

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Nature; 296(5860):811-816 1982

Numerous actin-binding proteins from a variety of cell types have been described. Here I attempt to correlate the properties and functions of some of these. Three major classes have been identified: (1) cross-linking proteins which form filament bundles or isotropic gels; (2) proteins which cap filament ends and nucleate the polymerization of G-actin (many of these also sever actin filaments); (3) proteins which bind to G-actin and stabilize the monomer pool. Some of the proteins described here combine the properties of more than one class and the activities of many of them are regulated by changes in Ca + + ion concentration. (Author abstract) (105 Refs)

10. QUANTITATION AND CHARACTERIZATION OF THE MICROTUBULE ASSOCIATED MAP2 IN PORCINE TISSUES AND ITS ISOLATION FROM PORCINE (PK15) AND HUMAN (HELA) CELL LINES.

Valdivia MM, Avila J, Coll J, Colaco C, Sandoval IV
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Biochem Biophys Res Commun; 105(4):1241-1249 1982

Microtubule polymerization-depolymerization protein (MAP2; av mol wt 300,000) was quantified and characterized from porcine tissues and cultured confluent pig kidney cells (PK15) and HeLa cells. MAP2 were demonstrated in all porcine tissues tested, with the highest levels in nervous tissue (9.7-11 ug/ml protein) and lower levels in non-nervous tissues (ranging from 104 nanog/ml protein in kidney to 9.7 nanog/ml protein in liver). MAP2 was associated with microtubules in cultured PK15 cells. With special emphasis on rapid isolation of the protein to avoid proteolysis, MAP2 was demonstrated in both PK15 cells and HeLa cells. The major protein isolated from PK15 cells was similar to brain MAP2 in mol wt of its two polypeptide components and the ability to promote polymerization of purified tubulin into microtubules. The HeLa protein retained by the MAP2 antibody-Protein A-Sepharose column contained a heat resistant polypeptide of lower mol wt (200,000 daltons) than brain MAP2. This HeLa protein did not induce tubulin polymerization. It is suggested that some cells may contain MAP2 related proteins rather than a protein identical to brain MAP2. (37 Refs)

11. MONOCLONAL ANTIBODIES THAT RECOGNIZE DISCRETE FORMS OF TUBULIN.

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Lab. Neural and Endocrine Regulation, Massachusetts Inst. Technology, Dept. Nutrition and Food Science, Cambridge, MA, 02139
Proc Natl Acad Sci USA; 79(8,part1):2579-2583 1982

Anti-tubulin antibodies secreted by plasmacytoma NSI-spleen cell hybrids were detected by an indirect binding assay. Different antibodies bound to different combinations of the tubulins as resolved by isoelectric focusing. Two monoclonal antibodies (TUB 2.1 and TUB 2.5) labeled only (i) the tubulin band on a polyacrylamide electropherogram and (ii) beta-tubulins as resolved by isoelectric focusing. The fraction that was specifically bound and eluted from antibody affinity columns was enriched in beta-tubulins as compared with alpha-tubulins, suggesting the possibility of some soluble tubulin homodimers and alpha, beta-heterodimers. Double labeling experiments were used to show that all detectable microtubules contained beta-tubulin. (Author abstract) (35 Refs)

12. TWO-DIMENSIONAL ANALYSIS OF HUMAN LYMPHOCYTE PROTEINS II. REGULATION OF LYMPHOCYTE PROTEINS BY A MOLECULE PRESENT IN NORMAL HUMAN URINE.

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Clin Chem; 28(4,part2):1074-1083 1982

Lymphocytes cultured with concentrated human urine develop many alterations. New proteins appear ('Urocon' proteins), while others disappear ('Urocof' proteins) from the two-dimensional gel pattern. This paper characterizes a human urinary leukocyte effector protein ('HULEP') this is responsible for the appearance of the Urocon:11-15 proteins in human lymphocytes. The effector molecule, HULEP-1, is a protein with a relative molecular mass in the range of 29,000 to 43,000. Urocon:11-15 production, cytoskeletal changes in the lymphocyte, and the presence of added HULEP-1 in the cultures are all directly related. This molecule appears to alter the structure of the lymphocyte by degrading actin and possibly other lymphocyte cytoskeletal proteins. Peptide maps of Urocon:11-15 and beta and lambda actin provide evidence that Urocon:11-15 proteins are closely related to nonmuscle actin, and may be degradation products of this major cytoskeletal protein. (Author abstract) (33 Refs)

13. CHANGES IN CONTRACTILE PROTEINS DURING DIFFERENTIATION OF MYELOID LEUKEMIA CELLS. II. PURIFICATION AND CHARACTERIZATION OF ACTIN.

Nagata K, Sagara J, Ichikawa Y
Dept. Cytochemistry, Chest Disease Res. Inst., Kyoto Univ., Kyoto 606, Japan
J Cell Biol; 93(2):470-478 1982

A myeloid leukemia cell line, M1, differentiates to macrophage and gains locomotive and phagocytic activity when incubated with conditioned medium (CM) from a fibroblast culture and bacterial endotoxin. To characterize the actin molecules before and after differentiation, the actin was purified through three sequential steps: DEAE-Sephadex A-50, polymerization/depolymerization, and Sephadex G-150 chromatography. The critical concentrations for polymerization were compared under different salt concentrations and temperatures. Results together with the data presented in our previous paper, the D- actin in the crude extract did not polymerize, suggest that an inhibitor for actin polymerization is present in this subline. The kinetics experiment revealed a slower polymerization of untreated M1 and D- cell actins as compared with CM-treated M1 actin. This delayed polymerization was due to a delay during the nucleation stage, not during the elongation stage. By isoelectric focusing, the ratios of beta- to gammaa-actin showed a marked difference depending on the states of cells. Tryptic peptide maps also revealed the presence of different peptides. Thus, the functional differences of actin before and after the differentiation was accompanied by some chemical changes in actin molecules. (61 Refs)

14. INDUCTION OF NEUROFILAMENT TRIPLET PROTEINS IN PC12 CELLS BY NERVE GROWTH FACTOR.

Lee V, Trojanowski JQ, Schlaepfer WW
Div. Neuropathology, Dept. Pathology, Univ. Pennsylvania Medical Sch., Lab. Medicine, Philadelphia, PA, 19104
Brain Res; 238(1):169-180 1982

Indirect immunofluorescence and monospecific, immunosorbent purified antibodies to 68,000, 150,000, and 200,000 dalton neurofilament proteins were used to localize neurofilament triplet proteins in PC12 cells grown in the absence of (PC12-) or in the presence of (PC12+) nerve growth factor (NGF). PC12-cells, a neoplastic counterpart of adrenal chromaffin cells, which resemble undifferentiated primitive sympathetic or 'blast' cells, had low levels of neurofilament proteins. Subsequent to being exposed to NGF, they became PC12+ cells and developed features observed in postmitotic sympathetic neurons. This transition from a pre- to postmitotic state coupled with neuronal differentiation also coincides with the induction of high levels of neurofilament proteins. Thus the induction of neurofilament proteins is another marker of neuronal differentiation in this cell line. However, the synthesis and induction of neurofilament proteins in PC12 cells deviated from that which occurs in the normal counterpart of PC12+ cells, ie, sympathetic neurons, since in PC12 cells they apparently do not assemble into intact neurofilaments. Failure of neurofilament proteins to assemble into intact neurofilaments indicates a complexity in neurofilament protein synthesis and assembly which has not been previously recognized or documented. It further indicates that the PC12 cell line is not only a useful model for studies of neuronal differentiation but also for studies of neurofilament synthesis, assembly, and ontogeny. (24 Refs)

15. ISOLATION, CHARACTERIZATION, AND SPONTANEOUS INTERCONVERSION OF TWO FORMS OF HUMAN PROSTATIC ACID PHOSPHATASE.

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Prostate; 3(2):165-181 1982

Human prostatic acid phosphatase, purified by affinity chromatography on Sepharose-bound N-(6-aminohexyl)tartramic acid, can be separated into two electrophoretically-distinguishable forms. While these two forms have similar amino acid compositions and kinetic properties, slight structural differences are revealed by circular dichroism and fluorescence spectroscopy. The two forms appear to differ primarily in the structure of their (nonphosphorylated) carbohydrate side chains. The spontaneous interconversion between the two forms under storage conditions may be important in evaluating the clinical significance of these enzyme forms in diagnosing prostatic cancer. (47 Refs)

16. VISUALIZATION OF THE FILAMENTOUS ORGANELLE (CYTOSKELETON) IN CULTURED CELLS WITH COOMASSIE BLUE R250.

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Dept. Cell Biology, Cancer Inst. Chinese, Acad. Medical Sciences, Beijing, People's Republic of China
Acta Anat Sin; 12(4):427-431 1981

The cytoskeleton of cells is mainly composed of microtubules and microfilaments, which has recently been used as a marker for cell transformation in vitro. Using the Coomassie blue stain, visualization of the cytoskeleton is described for cultured normal and tumor cells. In normal cells, such as murine heart cells and murine and human esophageal epithelial cells, cytoskeleton elements were observed as straight fibers running throughout the cell and parallel to the cell surface. In epithelial cells of human esophageal carcinoma (ECa109), cytoskeleton elements were few in number and arranged irregularly in a network. When subjected to cytochalasin D or heat (42.5 and 44.5 C), these filamentous elements became diffused. They aggregated into bundles when treated with colchicine or with a stem extract of Maytenus Hookerii Loas. During cell division, cells were seen to attach to other cells by protein elements. The relationship between cytoskeleton as visualized by Coomassie blue and actin as seen by immunofluorescence technics is discussed. (10 Refs) (In CHI)

17. ORNITHINE DECARBOXYLASE PROTEIN DIVERSITY AND ACTIVITY MODULATION IN HTC CELLS.

Mitchell JL, Mitchell GK
Dept. Biological Sciences, Northern Illinois Univ., DeKalb, IL, 60115
Biochem Biophys Res Commun; 105(3):1189-1197 1982

Multiple ionic forms of ornithine decarboxylase (ODC) were observed in isolated mammalian hepatoma tissue culture cells. The similarity of the three forms isolated with respect to apparent Km for pyridoxyl 5'-phosphate and ornithine indicates that these are most likely not separate gene products but rather reflect a minor alteration in the basic ODC protein. (16 Refs)

18. ANALYSIS OF POLYADENYLATE-PROTEIN COMPLEX OF POLYSOMAL MESSENGER RNA FROM MOUSE L CELLS.

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Hoppe Seylers Z Physiol Chem; 363(3):221-228 1982

The organization of polysomal poly(A)-ribonucleoprotein complex [poly(A)-RNP] was studied. The poly(A)-associated proteins were liberated directly from the poly(A)-RNP complex. Polysomal mRNA was isolated from L5178y mouse lymphoma cells by oligo(dT)-cellulose chromatography; poly(A)-RNP was prepared by nuclease digestion. The poly(A)-RNP fraction was considered to be pure basing on the size of its poly(A)component which was determined to consist of 155 AMP moieties. By radiolabeling with [3H]dansyl chloride, two poly(A)-associated proteins with molecular masses of 77,000 Da (P77) and 54,000 Da(P54) were identified. In a quantitative approach, it was shown that the polysomal poly(A)-RNP complex is composed of approximately four molecules P54 and two molecules P77. Digestion experiments with dimers and tetramers containing ribonuclease A indicated that P54 covers 15-20 AMP residues and P77 a sequence of 40-45 nucleotides on the poly(A)155 stretch of polysomal poly(A)-RNP. (Author abstract) (41 Refs)

19. TWO-DIMENSIONAL GEL ELECTROPHORESIS OF HUMAN BRAIN PROTEINS. II. SPECIFIC PROTEINS AND BRAIN SUBFRACTIONS.

Comings DE, Carraway NG, Pekkula-Flagan A
Dept. Medical Genetics, City of Hope Medical Center, Duarte, CA, 91010
Clin Chem; 28(4,part2):790-797 1982

Brain proteins extractable with distilled water or 9 mol/L urea were subjected to two-dimensional gel electrophoresis. They are examined in relation to the identification of actin, tubulin, neurofilament proteins, glial fibrillary acidic protein; proteins of human embryonic neocortex, synaptosomes, myelin, and plasma; and rat neocortex, rat glial, and mouse neuroblastoma cell lines. (Author abstract) (50 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

20. SELECTIVITY IN MATRIX COLLAGEN DEGRADATION BY TUMOR COLLAGENASES (MEETING ABSTRACT)

Dabbous MK, Haney L, El-Torky M, Brinkley SB
Department of Biochemistry, University of Tennessee Center for the Health Sciences, Memphis, TN, 38163
Fed Proc; 41(3):636 1982

Differences in susceptibility of the various genetically distinct collagen types to cleavage by collagenase has been observed. These differences may account for alterations in the collagenous connnective tissue matrix during physiologic and pathologic processes. Rabbit VX-2 carcinoma-derived cells released collagenase activity in serum-free media of monolayer cell cultures. The release of enzyme activity continued for 6-8 days and was subject to inhibition by cyclohexamide, thus, indicating that the enzyme activity was protein synthesis-dependent. The enzyme production increased as a function of passage number for six passages then decreased thereafter to a normal low level. Partial purification of the enzyme activity in the media by gel filtration on P-150 and Sepharose CL-6B columns demonstrated that certain chromatographic fractions showed selectivity in their action at 27 degrees on types I, III, IV and V collagens as evidenced by electrophoretic analysis of their cleavage products. The enzyme fraction which cleaved type IV collagen did not significantly alter the other collagen types. The enzyme fraction which cleaved type V collagen had limited effect on types I and III collagens but was without effect on type IV. Heterogeneity of the tumor collagenolytic activity with respect to substrate-type specificity, appears to provide an efficient mechanism for tumor growth, invasion and metastases. (no Refs)

21. COLLAGEN TRYPTIC PEPTIDES OF MINERALIZED AND NONMINERALIZED COLLAGEN FROM OSTEOBLASTOMA AND NORMAL BONE (MEETING ABSTRACT)

Banes AJ, Yamauchi M, Mechanic GL
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Fed Proc; 41(3):636 1982

Nonmineralized collagen (NMC) is considered to comprise the most recently formed collagen in bone. However, in bone disease, a greater proportion than normal of NMC may exist. Collagen left unmineralized may mature at a more rapid rate than that in the mineralized compartment. Collagen present in the NMC portion of bone has a different crosslink pattern compared with that of mineralized collagen (MC). Long bones from 4-week-old normal and osteoblastoma (AO) chicks were cleaned and powdered. Surface collagen was reduced with NaB3H4 and hydrolyzed with trypsin, and chromatographed on columns of G-50 superfine or reverse phase columns; or collagenase, and acid hydrolyzed and amino acids and crosslinks separated. The remaining pellet was demineralized, reduced with NaB3H4 and hydrolyzed as above. Results indicate that the crosslink pattern of normal NMC and MC are different: the former having a greater ratio of dihydroxylysinonorleucine/hydroxylysinonorleucine (DHLNL/HLNL). The pattern for osteoblastoma collagen indicates a similar trend, but even greater disparity between NMC and MC. The G-50 superfine and reverse phase HPLC (high pressure liquid chromatography) pattern for tryptic peptides of collagen from NMC show different patterns and more polar peptides than are present in MC for both normal and AO collagens. The results indicate that the NMC crosslink content and location differ from that of MC, and that bone disease can alter this profile further. (no Refs)

22. DIFFERENTIAL ATTACHMENT OF HIGH AND LOW METASTATIC FIBROSARCOMA CELLS TO TYPE IV COLLAGEN (MEETING ABSTRACT)

Varani J, Lovett EJ, Wicha M, Shibata S, Goldstein IJ
Department of Pathology, The University of Michigan Medical School, Ann Arbor, MI, 48109
Fed Proc; 41(3):336 1982

High and low metastatic tumor cell lines were isolated from a methylcholanthrene-induced murine fibrosarcoma. The lines were compared with regard to various parameters of adhesiveness. No significant differences were found in rates of attachment to plastic culture dishes or to Types I/III collagen matrices prepared from rat tail collagen. However, when a Type IV collagen matrix, prepared from EHS sarcoma was used, the high metastatic cells attached much more rapidly (and to a greater extent) than the low metastatic cells. When the Type IV collagen was pretreated with laminin, the attachment of the low metastatic cells was increased greatly. Very little enhancement of the high metastatic cells was induced by similar pretreatment. Laminin pretreatment did not enhance the attachment of either cell line to Types I/III collagen. It is suggested that the differences in metastatic ability between these line may be due, in part, to differences in attachment capabilities on Type IV collagen. Laminin may play a role in these differences. (no Refs)

23. ROLE OF LAMININ IN THE ATTACHMENT AND METASTASIS OF MURINE TUMOR CELLS.

Terranova VP, Liotta LA, Russo RG, Martin GR
Lab. Developmental Biology and Anomalies, Natl. Inst. Dental Res., NIH, Bethesda, MD, 20205
Cancer Res; 42(6):2265-2269 1982

We have studied the attachment of two murine metastatic cell lines and of a transformed, nonmetastatic sarcoma cell line to Type IV (basement membrane) collagen. The metastatic cells attached preferentially to Type IV collagen, whereas the nonmetastatic cells attached best to Type I collagen. Laminin increased both the rate and the number of metastatic cells attaching to Type IV collagen, while fibronectin had no effect. Antibodies to laminin prevented the attachment of metastatic cells to Type IV collagen, while antibodies to fibronectin prevented the attachment of the nonmetastatic cells. The number of pulmonary metastases which formed after iv injection of cells into C57BL mice was used to measure the metastatic propensity of these cell lines. A subpopulation of the metastatic cells selected for by their ability to attach to Type IV collagen in the presence of laminin produced more metastases than did unattached cells or cells attached with fibronectin. In addition, incubation of metastatic cells with antibody to laminin prior to injection into mice markedly reduced the number of lung metastases. These data suggest that laminin promotes the attachment of metastatic tumor cells to basement membrane during the metastatic process. (Author abstract) (31 Refs)

24. IMMUNOFLUORESCENCE LOCALIZATION OF FIBRONECTIN IN CHONDROSARCOMA CARTILAGE MATRIX.

Kimata K, Foidart JM, Pennypacker JP, Kleinman HK, Martin GR, Hewitt AT
(c/o Hewitt) Lab. Developmental Biology and Anomalies, Natl. Inst. Dental Res., NIH, Bethesda, MD, 20205
Cancer Res; 42(6):2384-2391 1982

In this study, we have compared the extracellular matrix components and the in vitro adhesion characteristics of normal rat epiphysial chondrocytes with those from the Swarm rat chondrosarcoma, which has many of the biochemical characteristics of normal cartilage. With the use of immunofluorescence techniques, tissue slices and chondrocytes in culture were tested for the presence of collagen Types I and II, cartilage-characteristic proteoglycan, and fibronectin. Both normal and tumor matrix contained Type II collagen and cartilage proteoglycan, but only the tumor matrix contained fibronectin. In culture, tumor-derived chondrocytes continued to accumulate fibronectin in their matrix, even after deposition of Type II collagen and proteoglycans, while normal chondrocytes did not. When the attachment characteristics of both types of chondrocytes were compared, tumor chondrocytes required fibronectin for attachment, while normal chondrocytes used another attachment factor that had been identified previously as chondronectin. These studies suggest that, although biochemically similar to normal chondrocytes, tumor chondrocytes are no longer able to express the regulatory mechanisms for fibronectin accumulation. (Author abstract) (44 Refs)

25. TUMOR-DERIVED CHEMOATTRACTANTS FOR FIBROBLASTS (MEETING ABSTRACT)

Gleiber WE, Schiffmann E
National Institutes of Health, Bethesda, MD, 20205
Fed Proc; 41(3):576 1982

The majority of human breast carcinomas are encapsulated by fibrous connective tissue apparently produced by the host in response to the growing tumor. Possibly, the tumor is producing an attractant bringing fibroblasts to the area around the tumor. Conditioned media from human mammary carcinoma cells maintained in vitro were assayed for fibroblast chemoattractant(s). A human breast tumor cell line, ZR-75-1, was cultured for 48 hours in a defined, serum-free medium containing insulin, transferrin, and estradiol. This conditioned medium was assayed for chemoattractant activity for fibroblasts using Boyden chambers. The conditioned medium was found to have potent chemotactic activity for normal human embryonic fibroblasts, CRL-1475, as indicated by a 'checkerboard' analysis. This activity is sensitive to protease digestion but resistant to RNase and DNase. The atractant is stable over a wide pH range (3 to 11) and to heating at 50C for one hour. Partial purification indicated a protein of 100-150,000 daltons based on molecular sieve chromatography. When tested for its effect upon other cell types, the conditioned medium strongly attracted both endothelial and smooth muscle cells, but inhibited the chemotactic response and neutrophils toward the attractant F-Met-Leu-Phe. Conditioned media from three other human breast tumor cell lines also contained fibroblast attractants. These studies may give insight into the mechanism of the fibrotic involvement in various tumors such as breast carcinomas. (no Refs)

26. TWO TYPES OF PLASMINOGEN ACTIVATORS SECRETED BY MCF-7 CELLS.

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Biochem Biophys Res Commun; 105(4):1597-1604 1982

Proteases secreted by MCF-7 breast cancer cells were investigated with affinity chromatography to distinguish between plasminogen-dependent and -independent proteases. Four of the proteases degraded casein in the absence of plasminogen, had mol wt of 120,000, 90,000, 87,000 and 82,000 daltons, and three were retained on a p-aminobenzamidine-Sepharose affinity column, suggesting that they were trypsin-like proteases. None of these were retained on the antiurokinase column. Two plasminogen activators (PA: 59,000 and 37,000-39,000 daltons) identified in the unfractionated medium were retained on p-aminobenzamidine-Sepharose, and a small amount of PA of mol wt 65,000 was distinguishable from the major band. The antiurokinase-bound fraction contained two prominent PA of mol wt 65,000 and 39,000 and two minor PA of 31,000 and 28,000 daltons. Sodium dodecyl sulfate gel electrophoresis of the antiurokinaese-bound fraction demonstrated two bands of 55,000 and 30,000 daltons which were identified as glycoproteins. Secreted proteases in serum-free conditioned medium during MCF-7 growth included nonactivator proteases (mol wt 120,000, 90,000, 87,000, and 82,000). Prominent plasminogen-dependent proteolysis was observed with 65,000, 59,000, and 37,000-39,000 dalton molecules. The 59,000 dalton PA, while associated with p-aminobenzamide Sepharose, was not observed in the eluate of the antiurokinase Sepharose column. These findings suggest that the presence of two distinctly different molecular species of activators may be important in studies of the regulation of PA synthesis and/or activity with MCF-7 cells. (33 Refs)

27. PURIFICATION OF AN IMMUNOREGULATORY FACTOR RELEASED BY MALIGNANT PLASMA CELLS (MEETING ABSTRACT)

Ullrich S, Zolla-Pazner S
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Fed Proc; 41(3):480 1982

It has been demonstrated previously that the immunosuppression observed in plasmacytoma-bearing mice is the result of a cascade of events involving soluble immunoregulatory factors and host macrophages. Malignant plasma cells release a lymphokine, termed PC-factor, which interacts with normal host macrophages, resulting in the release of a second soluble factor which causes the suppression of antigen-stimulated antibody production. The studies described here provide a methodology for the purification of PC-factor, the mediator which initiates this suppressive cascade. An in vitro cloned line of the murine plasmacytoma MPC-11, designated 456,31, was used as a source of PC-factor. The spent culture fluid was subjected to ammonium sulfate fractionation and chromatography on Sephacryl 200, DEAE (diethylaminoethyl)-cellulose and Protein A- Sepharose. The active fraction thereby obtained was further purified by high pressure liquid chromatography and eluted as a homogeneous peak. Thus PC-factor was shown to be a non-immunoglobulin protein which migrates with a molecular weight of approximately 55,000. The biologic activity is retained after heating at 56 degrees for 30 min and after repeated cycles of freezing and thawing. The ability to purify PC-factor should facilitate future studies into the biologic and biochemical mechanisms which are responsible for immunoregulation in myeloma. (no Refs)

28. COMPLETE AMINO ACID SEQUENCE OF THE DELTA HEAVY CHAIN OF HUMAN IMMUNOGLOBULIN D.

Takahashi N, Tetaert D, Debuire B, Lin LC, Putnam FW
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Proc Natl Acad Sci USA; 79(9):2850-2854 1982

We have determined the amino acid sequence of the variable (V) region of the delta heavy (H) chain of human IgD isolated from the plasma of myeloma patient WAH. This V region is usually in its amino end group (arginine) and in its length (129 residues). The length is due to 10 insertions in the third complementarity-determining region (CDR3). A computer search showed that no reported CDR3-joining region (-JH) sequences are identical and that they appear to be unrelated to the constant (C) region sequences of immunoglobulins. The V region sequence together with our previous results for the C region give the complete sequence of the human delta chain WAH, which has 512 amino acid residues and a Mr approx = 65,000. The human delta chain has four domains (V, Cdelta1, Cdelta2, and Cdelta3) and a long hinge region; by comparison, the mouse delta chain lacks a continuous segment of 135 residues, including half the hinge region and the entire Cdelta2 domain. The human and mouse delta chains also differ in the number, kind, and location of GlcN and GalN glycans and probably in conformation and quaternary structure. These and other considerations suggest that there may be multiple forms of both secreted and membrane-bound IgD that differ in size, structure, and function. (Author abstract) (34 Refs)

29. IMMUNOASSAY OF FERRITIN IN PLASMA.

Halliday JW
Dept. Medicine, Univ. Queensland, Royal Brisbane Hosp., Herton 4029, Australia
Methods Enzymol; 84:148-171 1982

Ferritin (FR), isoferritins (IFR), immunoassays, purification of tissue FR, storage of FR, criteria for purity of FR, preparation of antiserum to human tissue FR, measurements of FR protein concentration, preparation of purified antiferritin antibodies, iodination procedures, FR assay techniques, and effects of IFR composition on FR assays are discussed. IFR of normal human heart and HeLa cells are primarily of the more acidic type and have been shown to differ in immunological reactivity from FR of liver and spleen. Because acidic IFR have been detected in some tumors, assays for the more acidic IFR have been developed. (44 Refs)

30. A MONOCLONAL ANTIBODY SPECIFIC FOR A DISTINCT REGION OF HEN EGG-WHITE LYSOZYME.

Kobayashi T, Fujio H, Kondo K, Dohi Y, Hirayama A, Takagaki Y, Kosaki G, Amano T
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Mol Immunol; 19(4):619-630 1982

Six stable hybrid cell lines that secrete homogeneous anti-hen egg-white lysozyme (HEL) antibodies were prepared by cell fusion between cells of immunoglobulin non-synthetizing myeloma (SP2/0-Ag14) and spleens of A/J mice. One (Hyb - C1) of these hybridoma antibodies was found to be specific for the N- and C-terminal portions of HEL. It showed restricted heterogeneity on isoelectrofocusing and an isoelectric point of about pH 6.0. The heavy- and light-chain classes of the Hyb - C1 antibody were gamma1 and kappa, respectively. The association constant of the Hyb - C1 antibody with HEL at 37 C was 3.3 x 10(7) (L/M). The Hyb - C1 antibody was further characterized by its cross reactivities with other avian lysozymes. The association constants (KIs) of cross-reactive avian egg-white lysozymes at 37 C, calculated from the inhibitions of binding of 125I-HEL to Hyb - C1 antibody were 3.2 x 10(7) (L/M) for both Japanese quail lysozyme (JEL) and turkey lysozyme (TEL) and 1.2 x 10(6) (L/M) for two duck lysozymes (DL-1 and DL-2). The partial N-terminal amino acid sequences of the heavy and light chains of Hyb - C1 antibody up to the 25th residues were also determined. (Author abstract) (47 Refs)

31. EVIDENCE FOR A NOVEL CIRCULATING ALPHA1 PROTEIN IN TUMOR-BEARING RATS. DIFFERENTIATION FROM ACUTE PHASE ALPHA1 PROTEINS AND FROM ALPHA1 FETOPROTEIN.

Herve F, Urios P, Grigorova AM, Cittanova N, Rondeau Y, Engler R, Broadhead G, Neish W
Equipe de Recherche No. 239, Centre National de la Recherche Scientifique, Universite Paris V., Paris, France
Biochem Biophys Res Commun; 105(3):861-868 1982

The isolation of a novel alpha1 protein (AP) from an experimental RD/3 rat sarcoma is described, and evidence that it is molecularly distinct from both acute phase AP and alphafetoprotein is presented. This new protein component may be linked to the development of the RD/3 tumor and may correspond to a tumoral protein. The presence of this protein may play a role in the modification of the transport properties of animals bearing an RD/3 tumor. (14 Refs)

32. PROTEINS OF HUMAN URINE. III. IDENTIFICATION AND TWO-DIMENSIONAL ELECTROPHORETIC MAP POSITIONS OF SOME MAJOR URINARY PROTEINS.

Edwards JJ, Tollaksen SL, Anderson NG
Dept. Pathology, Univ. North Carolina Medical Sch., Chapel Hill, NC, 27514
Clin Chem; 28(4,part2):941-948 1982

We mapped the proteins of human urine by high-resolution two-dimensional electrophoresis, utilizing the ISO-DALT system. Wide-range pH gradients and narrow-range acid gradients were both used in the first-dimension separations. The patterns revealed proteins ranging in relative molecular mass from 10,000 to 90,000. Proteins identified in the map included transferrin, albumin, hemopexin, alpha2-HS glycoprotein, alpha1-antitrypsin, Gc globulin, alpha1-acid glycoprotein, Zn alpha2-glycoprotein, retinol binding protein, beta2-microglobulin, the immunoglobulin light chains, and MAUP (most acid urinary protein). The use and utility of internal-charge and molecular-mass standards are described. We used electrophoretic transfer of proteins to nitrocellulose sheets and subsequent detection by immunological methods to identify some proteins. (Author abstract) (28 Refs)

33. ENHANCEMENT TECHNIQUES FOR DETECTING TRACE AND FLUID-SPECIFIC COMPONENTS IN TWO-DIMENSIONAL ELECTROPHORESIS PATTERNS.

Dermer GB, Silverman LM, Chapman JF
Dept. Pathology and Biochemistry, Univ. North Carolina Sch. Medicine, Chapel Hill, NC, 27514
Clin Chem; 28(4,part2):759-765 1982

Albumin and other serum-derived proteins were removed from several types of body fluids by affinity chromatography, to facilitate detection of trace or non-serum-derived proteins in two-dimensional electrophoresis patterns. Albumin was removed by the dye Cibacron Blue F3G-A coupled to Sepharose. Two-dimensional patterns of albumin-depleted serum lack the large albumin spot, and several families of spots become visible that ordinarily are partly or totally hidden by it. However, other proteins also bind to Cibacron Blue. Most serum proteins, including albumin, were effectively removed by anti-human serum antibodies coupled to Sepharose. Two-dimensional patterns of serum-depleted cerebrospinal fluid exhibit five clusters of probable nervous-system protein families not detected in serum. One additional family, probably antigenically related to transferrin, was removed by the affinity step. Two-dimensional patterns of serum-depleted prostatic fluid exhibit five major non-serum families, two of which may be creatine kinase B subunits and prostatic acid phosphatase. Two-dimensional patterns of serum-depleted malignant effusions exhibit one or more of three proteins that possibly are tumor products. Pattern matching suggests the presence of one non-serum-derived protein family common to cerebrospinal fluid, prostatic fluid, and malignant effusions. Prostatic fluid and malignant effusions have in common as many as three non-serum families of proteins. (Author abstract) (22 Refs)

34. MITOGEN-INDUCED BLASTOGENESIS AND RECEPTOR MOBILITY INHIBITION BY BREAST CANCER SERUM WITH ELEVATED OROSOMUCOID (ALPHA1-ACID GLYCOPROTEIN) LEVELS.

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JNCI; 68(5):779-783 1982

Sera from cancer patients have been shown to suppress normal lymphoid cell responsiveness in vitro. In the present study, sera from breast cancer patients were demonstrated to be inhibitory to the concanavalin A (Con A)-, Proteus vulgaris-derived phytohemagglutinin-, and pokeweed mitogen-induced blastogenic responses of normal lymphoid cells. Orosomucoid (OR) (alpha1-acid glycoprotein), an acute-phase reactant, was elevated in these sera, and a positive correlation existed between the OR level in the sera and its immunosuppressive capacity. When normal lymphoid cells were reacted in fluorescein isothiocyanate-labeled Con A, cells that had been preincubated in breast cancer sera known to contain an elevated level of OR showed a significant decrease in Con A receptor mobility as compared to the receptor mobility of the same lymphoid cells preincubated in normal sera. Thus a component(s) from the sera of breast cancer patients had the capacity to inhibit lymphocyte activation. This inhibition might have resulted from an interaction of OR with the cell membrane. (Author abstract) (34 Refs)

35. STUDIES ON IMMUNITY IN PATIENTS WITH UROGENITAL CANCERS. REPORT 2. EFFECT OF ABNORMAL SERUM PROTEINS ON NONSPECIFIC CELLULAR IMMUNITY.

Ono H
Dept. Urology, Shimane Medical Univ., Izumo, Japan
Nippon Hinyokika Gakkai Zasshi; 72(8):1078-1091 1981

Subfraction levels of prealbumin (PA), alpha1-antitrypsin (a1-AT), alpha1-acidglycoprotein (a1-AG), ceruloplasmin (Cp) and haptoglobin (Hp) were measured in the serum of 66 patients with bladder carcinoma, 16 with renal cell carcinoma, 21 with pelvic and ureteric carcinoma, 19 with prostatic carcinoma, 5 with testicular tumor, 15 with other malignancies, 22 with benign prostatic hypertrophy, and 15 with no malignancy. Effects of the serum proteins on nonspecific cellular immunity was studied. Skin response to 2-4 dinitrochlorobenzene (DNCB) and purified protein derivative (PPD) and lymphocyte blastogenesis induced by phytohemagglutinin (PHA) and pokeweed mitogen (PWM) were used as indicators of immune response. Alpha1-globulins were significantly increased in patients with metastatic renal carcinoma, pelvic and ureteric carcinoma, or other malignant tumors, and the increases were directly related to the stage of the cancer. High levels of IgG and IgM were observed in the patients with renal cancer but not in other patients. Patients with urinary tract cancers had elevated levels of PA, a1-AG, a1-AT, Cp, and Hp; a1-AT was significantly increased in patients with advanced cancers. Decreased response to PPD was associated with significant decreases in PA and increases in a1-AT, Cp, and Hp. Except for PA and a1-AG, no other serum proteins were associated with changes in DNCB responses. After the addition of autologous serum, PHA-induced blastogenic response was positively correlated with levels of serum PA and negatively correlated with levels of serum a1-AG, a1-AT, and Cp. PWM-induced response was not correlated to levels of serum glycoproteins. The patient's lymphocyte reactivity index was negatively correlated to a1-AT and Cp levels, suggesting suppression of T lymphocyte function by these glycoproteins. (27 Refs) (In JPN)

36. STIMULATION OF LIVER CELL DNA SYNTHESIS BY ONCOMODULIN, AN 11,500 MW CALCIUM-BINDING PROTEIN FROM HEPATOMA.

Boynton AL, MacManus JP, Whitfield JF
Animal and Cell Physiology Group, Div. Biological Sciences, Natl. Res. Council of Canada, Ottawa, K1A OR6, Canada
Exp Cell Res; 138(2):454-458 1982

Raising the calcium concentration, or adding the tumor-specific calcium-binding protein oncomodulin (but not a similar, calcium-binding protein such as skeletal muscle parvalbumin) stimulated DNA synthesis in non-neoplastic T51B rat liver cells, whose DNA-synthetic activity had been reduced by incubation in medium containing 0.02 mM calcium instead of the usual 1.8 mM calcium. A calcium:oncomodulin complex was probably the actual stimulator, because oncomodulin action was blocked by further reducing the ionic calcium concentration in the already calcium-deficient medium with EGTA. Oncomodulin was also able to stimulate DNA synthesis in T51B cell cultures, whose response to calcium addition had been blocked by trifluoperazine. (Author abstract) (32 Refs)

BINDING/ RECEPTOR PROTEINS

37. ENHANCED BINDING OF RETINOIC ACID TO ITS CELLULAR BINDING PROTEIN IN LEWIS LUNG TUMOR CYTOSOL BY ALKALINE PHOSPHATASE TREATMENT.

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Hoppe Seylers Z Physiol Chem; 363(3):337-340 1982

It is suggested that the action of retinoids is mediated by specific binding proteins. Such proteins for retinol and retinoic acid were isolated and characterized from several tissues. Retinoids might act in a manner similar to that known for steroid hormones. There is some evidence that the activity of steroid hormone receptors is influenced by phosphorylating-dephosphorylating processes. Here we report the influence of alkaline phosphatase on the specific binding of retinoic acid to Lewis lung tumor cytosol. (Author abstract) (15 Refs)

38. CHARACTERIZATION OF CELLULAR RETINOID-BINDING PROTEINS FROM FETAL RABBIT TISSUES BY HIGH-PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY (MEETING ABSTRACT)

Rainier S, Herrara JM, McCormick AM
University of Texas Health Science Center, Dallas, TX, 75235
Fed Proc; 41(3):682 1982

A rapid high-performance size exclusion chromatography (HPSEC) method was developed for the characterization of cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP). The results obtained by HPSEC are comparable to those obtained by sucrose density gradient analysis of CRBP and CRABP. HPSEC offers the advantages of reduced analysis time and decreased dissociation of ligand-protein complexes during their characterization. In addition, the HPSEC method provides good resolution of CRBP and CRABP from non-specific retinoid-binding components of cytosol. Thus, HPSEC permits the simultaneous characterization of cellular retinoid-binding proteins and determination of the specific binding capacity of various tissues. This method was used to characterize CRBP and CRABP in fetal rabbit tissues. Significant amounts of a specific retinol-binding component were found in kidney, brain and intestine. A specific retinoic acid-binding component was also found in significant amounts in kidney, brain and intestine. Enzyme studies were used to establish the protein nature of these cellular retinol and retinoic acid-binding components. The molecular weights of CRBP and GRABP detected in fetal rabbit tissues are comparable to those described in adult and fetal rat tissues. (no Refs)

39. RETINOIC ACID BINDING PROTEIN IN NORMAL AND NEOPLASTIC RAT PROSTATE.

Gesell MS, Brandes MJ, Arnold EA, Isaacs JT, Ueda H, Millan JC, Brandes D
(c/o Isaacs) James Buchanan Brady Urological Inst., Johns Hopkins Hosp., Marburg Building, 600 N. Wolfe St., Baltimore, MD, 21205
Prostate; 3(2):131-138 1982

Cytosolic retinoic acid-binding protein (cRABP) was detected, using sucrose density gradient centrifugation, in four sublines of the Dunning R 3327 transplantable prostatic carcinoma and in the normal lateral prostate of the Fisher-Copenhagen F1 rat; cRABP was not detected in the dorsal lobes (the tissue of origin of R 3327 in a Copenhagen rat) or ventral prostate. The presence of cRABP in all four R 3327 tumor sublines, which differ in their degree of differentiation and hormone responsiveness, suggests that cRAPB may be a marker of malignant transformation similar to other oncofetal proteins in human and animal tumors. (40 Refs)

40. STUDIES ON MANNOSYL CARRIER FUNCTION OF RETINOL AND RETINOIC ACID IN EPITHELIAL AND MESENCHYMAL TISSUES.

De Luca LM
Differentiation Control Section, NCI, Bethesda, MD
J Am Acad Dermatol; 6(4,part2):611-619 1982

Mannosyllretinylphosphate (MRP) acts as a carrier of mannose, mostly in a direct transfer to protein, in the complex biosynthetic mechanism responsible for glycoprotein assembly in mammalian membranes. Five double bonds seem to be required for this function of MRP. In liver and tracheal tissues from vitamin A-deficient hamsters and rats, retinoic acid (RA) and retinol (ROL) were equally effective in normalizing the in vivo incorporation of mannose into glycoproteins within a short time. RA also reversibly increased the adhesive properties of a spontaneously transformed mouse dermal fibroblast cell line (3T12 cells). A free carboxyl group seemed to be required for this activity of RA. Both RA and ROL enhanced the adhesiveness of 3T12 cells, but only the cellular RA-binding protein (RA-BP) was detected, suggesting that the cellular ROL-binding protein (ROL-BP) may not be a requirement for this activity. The 3T12 cells contained a metabolite of RA (compound X), different from ROL, incorporated into a mannosylretinoidphosphate (MXP). This derivative comprised up to 40% of the total pool of 3H-mannose and 14C-RA derivatives synthesized after 48 hr of labeling. The chromatographic properties of this MXP were identical to those of MRP, but the retinoid in MXP (compound X) differed from both ROL and RA. The results suggested that ROL (in the form of MRP) and RA (in the form of MXP) may function as mannosyl carriers in biologic membranes. (27 Refs)

41. RETINOID-BINDING PROTEINS.

Goodman DS
Div. Metabolism and Nutrition, Dept. Medicine, Columbia Univ. Coll. Physicians and Surgeons, New York, NY, 10032
J Am Acad Dermatol; 6(4,part2):583-590 1982

Plasma retinol binding protein (PRBP), cellular retinol binding protein (CRBP), and cellular retinoic acid binding protein (CRABP) are immunologically distinct from each other. PRBP, produced in the liver and catabolized mainly in the kidneys, is responsible for the delivery of retinol to its extrahepatic sites of action. No disease has yet been identified in which PRBP was totally absent or immunologically abnormal. The results of preliminary experiments suggest that CRBP may be identical in different tissues and different species. Comparable information is not yet available for CRABP. The tissue levels and distributions of CRBP and CRABP in animal and human tissues are distinct from each other. Little is known about the factors influencing and regulating the tissue levels and metabolism of CRBP and CRABP. Retinoid-binding proteins (notably CRABP) have been identified in many tumors and cell lines. It has been reported that the binding ability of synthetic retinoids for the intracellular binding proteins (notably CRABP) tends to correlate with the ability of the retinoid to influence epithelial cell differentiation and/or tumor growth in vivo or in vitro. Further research is needed to define the biochemical processes mediated by these substances. (57 Refs)

42. RETINOIC ACID BINDING PROTEINS AND STEROID RECEPTOR LEVELS IN HUMAN BREAST CANCER.

Mehta RG, Kute TE, Hopkins M, Moon RC
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Eur J Cancer Clin Oncol; 18(3):221-226 1982

Samples of 31 human breast cancer tissues were examined for the presence or absence of cytosolic retinoic acid binding proteins (cRABP), estrogen receptors (ER) and progesterone receptors (PR). cRABP were detected as specifically sedimenting 2S components on sucrose density gradients. Specific binding under the 2S region for cRABP and under the 8S region for ER and PR were determined. The levels of cRABP ranged from less than 1 pmol to 14 pmol per mg of cytosol protein. Tumors containing less than 3 pmol cRABP were designated as low cRABP; tissues were considered ER- and PR- when the receptor levels were less than 10 fmol/mg protein. Approx 60% of primary and metastatic tumors examined contained high cRABP. Tumors with well differentiated histopathology exhibited increased levels of cRABP. There was no significant correlation between the presence or absence of steroid receptors and cRABP in human mammary tumors. (Author abstract) (25 Refs)

43. STUDY OF IGE AND IGG FC SURFACE RECEPTORS ON MOUSE MASTOCYTOMA CELLS (MEETING ABSTRACT)

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National Institutes of Health, Bethesda, MD, 20205
Fed Proc; 41(3):825 1982

The mastocytoma cell line MMC-1 derived fr[illegible] tumor in CXBI mice releases histamine with both IgE an[illegible]G mediated reactions. The nature of the Fc(epsilon) and Fc(gamma) receptors on these cells was investigated. To study the Fc(gamma) receptor we used a rabbit antiserum of the IgG2b receptor on the mouse macrophage P388D cell line. The Fc(epsilon) receptors were assayed using either rat IgE or rabbit antiserum to the Fc(epsilon) receptor of rat basophil leukemia cells. Fluorescent studies demonstrated simultaneous binding of rat IgE and antiserum to Fc(gamma) receptors, thus a single cell expressed both Fc(epsilon) and Fc(gamma) cell surface receptors. Cells were surface labeled with 125I, the membranes solubilized, and the Fc-IgG and Fc-IgE receptors immunoprecipitated and analyzed on sodium dodecyl sulfate polyacrylamide gels. IgE and anti-IgE or rabbit anti-Fc (epsilon) precipitated a broad band of protein with an apparent molecular weight of approx 60K. Anti-Fc-IgG antiserum precipitated a similar protein peak with a slightly higher apparent molecular weight (68-74K). By sequential immunoprecipitation, removal of the membrane component(s) reacting with rat IgE or antiserum to the Fc(epsilon) receptor left behind a component(s) precipitating with anti-Fc(gamma). Therefore, the FC(epsilon) and Fc(gamma) receptors are antigenically distinquishable and appear to be separate cell membrane components. (no Refs)

44. KINETICS OF INTERNALIZATION AND RECYCLING OF THE ASIALOGLYCOPROTEIN RECEPTOR IN A HEPATOMA CELL LINE.

Schwartz AL, Fridovich SE, Lodish HF
Dept. Biology, Massachusetts Inst. Technology, Cambridge, MA, 02139

J Biol Chem; 257(8):4230-4237 1982

The presence of a large number of functional asialoglycoprotein receptors in the human hepatoma cell line Hep G2 has allowed us to determine the parameters which define ligand binding to the surface receptor, ligand receptor internalization, and receptor recycling. We have developed a simple kinetic model which encompasses all of these processes. These cells contain 225,000 functional asialoglycoprotein receptors, and at 37 C uptake at a half-saturating concentration (2 ug/ml) of 125I-asialoorosomucoid (ASOR) is linear for several hr at a rate of 15,000 molecules/cell/min. Neither the rate of 125I-ASOR binding at 4 C nor the rate of uptake at 37 C was altered under conditions in which protein synthesis was completely inhibited. Thus each receptor recycles a ligand, on the av, every 16 min. The rate of binding of 125I-ASOR to cells at 37 C, independent of internalization, was measured in the presence of 10-12 mM azide. The rate of internalization at 37 C was determined from cells, prebound with ligand at 4 C, by following EDTA-resistant cell-associated 125I-ASOR. The distribution of functional receptors within the cell was assessed from ligand binding at 4 C and ligand uptake at 37 C in cells pretreated with trypsin to remove all surface receptors. In growing Hep G2 cells 87% of the receptors are on the cell surface, and over 88% of these are unoccupied with ligand. During steady state internalization of 125I-ASOR, the number of surface ASOR receptors decreases 17%. At 2 ug/ml of 125I-ASOR, an unoccupied surface receptor requires a mean time of 8.7 min to bind a ligand, and a mean time of 2.2-2.3 min is required to internalize a surface receptor-ligand complex. An additional 4.2 min is required for ligand to dissociate from an internalized receptor and for the receptor to return to the cell surface. We have been able to determine or calculate most of these parameters of receptor recycling by two or more independent sets of measurements. (Author abstract) (24 Refs)

45. ZINC, METALLOTHIONEIN, AND EHRLICH CELL PROLIFERATION (MEETING ABSTRACT)

Petering DH, Krakower G, Bachoski G, Shaw CF
University of Wisconsin-Milwaukee, Milwaukee, WI, 53201

Fed Proc; 41(3):641 1982

Ehrlich cell proliferation is inhibited by nutrient zinc deficiency. Zn (zinc) deficient, Zn(-), cells contain 75 to 100% of the normal, Zn(+), level of Zn. Activities of the Zn proteins, DNA and RNA polymerase, are much less affected than levels of thymidine kinase, not a known Zn metalloprotein. Zn(+) cells contain a protein which binds 20% of the cytosolic Zn. It is identified as a metallothionein (Mt) by its apparent molecular weight, exclusive binding of Cd, lack of aromatic amino acids, high SH content, and isolation as two isoproteins by DEAE (diethylaminoethyl) chromatography. In Zn(-) cells, no Zn in bound to Mt. Exposure of cells to Cd inhibits proliferation similarly to Zn deficiency as described above. At least 85% of the cellular Cd is in Mt. Treatment of cells with a bis(thiosemicarbazonato) Cu(II) complex places Cu exclusively into Mt at concentrations which inhibit cell proliferation. These results suggest that Zn-Mt is critical for Ehrlich cell proliferation. According to model studies with rat liver Mt, the protein may be a specific Zn donor to apo-Zn structures. Zn-Mt can donate Zn rapidly to apocarbonic anhydrase. Zn in Mt also appears to be thermodynamically accessible to ligands with intermediate binding affinity for zinc (log K less than 10); a bis(thiosemicarbazone) with log K -9.7 can completely remove Zn from metallothionein. (no Refs)

46. IDENTIFICATION AND PURIFICATION OF A RECEPTOR FOR TUMOR ASSOCIATED FETAL ANTIGENS (MEETING ABSTRACT)

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Fed Proc; 41(3):414 1982

Tumor associated fetal antigens (TAFA) are immunosuppressive low molecular weight proteins that share many molecular properties with interleukin-1. TAFA have pI (isoelectric point) heterogeneity; a molecular weight (MW) range of 12,000-17,000; and TAFA have been shown to suppress human and murine natural killer cell activity. In the present study, binding by TAFA-II to NBR rat spleen cells was shown to be reversible and saturable; and at equilibrium, affinity measurements revealed a moderate binding association constant of 2.4 x 10(6)/M. Normal spleen cells were treated with 125I-TAFA-II and then solubilized with 0.5% NP-40. The labeled and solubilized membrane extracts were analyzed by G-100 Sephadex chromatography. A complex with a MW of 90,000 was isolated and analyzed by isoelectric focusing. The 125I-TAFA-II/receptor complex was next immuneprecipitated with anti-TAFA-II. In the presence of TAFA-II and anti-TAFA-II, the purified receptor was immuneprecipitated and analyzed in 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Analysis of the dissociated complexes demonstrated the presence of a purified receptor (MW 65,000-75,000d). Using lentil lectin chromatography the receptor was found to be a mannose containing glycoprotein. These data indicate that receptor binding by TAFA is specific, and that the immunoregulatory effects of TAFA depends on direct interactions with immunocompetent cells. (no Refs)

47. A SPHINGOMYELIN TRANSFER PROTEIN IN RAT TUMORS AND FETAL LIVER.

Dyatlovitskaya EV, Timofeeva NG, Yakimenko EF, Barsukov LI, Muzya GI, Bergelson LD

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Eur J Biochem; 123(2):311-315 1982

Affinity resins containing covalently bound phospholipids were used to compare the affinity of the phosphatidylcholine transfer protein from beef liver and a low-specificity lipid transfer protein from rat hepatoma 27 to phosphatidylcholine and sphingomyelin. Binding experiments demonstrated that the beef liver protein associates specifically with immobilized phosphatidylcholine whereas the hepatoma protein showed a preference for sphingomyelin. Purified antiserum raised against the hepatoma sphingomyelin transfer protein was used to determine the presence of that protein in the cytosol of various experimental tumors as well as in those of normal, regenerating and fetal rat liver. The protein was found to be well expressed in all the tumors examined and in fetal liver as determined by immunodiffusion whereas only minute amounts could be detected in normal liver and in 30-h regenerated liver. The presence of the sphingomyelin transfer protein in cytosol was in parallel with the presence of sphingomyelin in the corresponding mitochondria. It is suggested that the occurrence of sphingomyelin in tumor and fetal liver mitochondria may be due to protein-catalyzed sphingomyelin transfer from the endoplasmic reticulum. (Author abstract) (33 Refs)

48. DIURNAL VARIATIONS IN COBALAMIN BINDING PLASMA PROTEINS.

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Scand J Haematol; 28(2):141-144 1982

The diurnal variations in cobalamin-binding plasma proteins were studied in 10 healthy volunteers. The unsaturated cobalamin-binding capacities of transcobalamins (TC-II and R-binders), P-cobalamin and P-protein were measured every 3rd hr during 24 hr. Only small variations within an interval of +-20% were found. Highly significant decrements during the night were a uniform finding. After correction for variations of total plasma protein, the changes became less prominent, but still significant for P-cobalamin and P-R-binders. Hence the mean values of P-R-binders and P-cobalamin were 113 and 533 pmol/l at 9:00 hr, and decreased 12 and 8%, respectively, at 3:00 hr. When using cobalamin binding plasma proteins in the monitoring of various malignant, hematological and autoimmune disorders, the specimens should, therefore, be collected at defined hours and in a uniform posture. (Author abstract) (15 Refs)

MEMBRANE/ SURFACE PROTEINS

49. SUGAR CHAINS ON LECTIN-BINDING GLYCOPROTEINS FROM METASTATIC MURINE B16 MELANOMA SUBLINES (MEETING ABSTRACT)

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Fed Proc; 41(3):1159 1982

Cell surface glycoproteins of metastatic murine melanoma cells (B16-F1, -F10, -BL6, -O13) are heterogenous and their carbohydrate moieties only partially elucidated. We have studied the sugar chains of the individual glycoproteins after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The method uses a combination of chemical modification of the sugar moieties and lectin staining to gels. Wheat germ agglutinin (WGA) bound to 60-67K, 80K, 93K, 115K and several minor components. After removal of sialic acid from the glycoproteins, WGA binding was no longer detected indicating that sialic acid is responsible for the binding. After disialization Arachis hypogaea agglutinin bound to 51K, 55K and 60-67K components, but only weakly to the 80K component; whereas Ricinus communis agglutinin-I bound almost identically to WGA. These latter lectins did not bind prior to removal of sialic acid. The results suggest that high molecular weight WGA receptors do not contain mucin-type but rather asparagine-linked complex-type sugar chains with high sialic acid contents. When the desialized non-WGA binding proteins were subjected to Smith periodate degradation, they regained the ability to bind to WGA. Standard glycoproteins with tribranched, complex-type sugar chains gave similar results suggesting the existence of such carbohydrate chains on WGA receptors. Relatively small differences in lectin binding were observed among B16 sublines with various organ colonizing capacities. (no Refs)

50. LECTIN-INDUCED REARRANGEMENT OF AN IMMATURE HEMATOPOIETIC CELL SURFACE MARKER.

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J Cell Physiol; 110(3):245-248 1982

The transformed murine stem cell line 416B was treated with both fluorescein-conjugated Con A (flCon A) and the dye merocyanine 540 (MC540). Binding regions for MC540 and receptors for flCon A were found to occur on the same cell surface areas. It was suggested that the observed association between liquid-like membrane domains and surface glycoproteins may be strong enough to permit reorganization of disordered lipid regions by forces affecting the proteins associated with them. It was also shown that capping of one membrane receptor resulted in the simultaneous capping of a second. It was suggested that sugar residues recognized by these two lectins are found on the same surface protein in 416B cells. If so, the two sugar residues must be relatively distant from one another on the protein molecule, since treatment with one at a concentration sufficient to induce cross-linking does not sterically inhibit the binding of the other. Alternatively, the two lectin receptors may be on separate molecules, with their common association with membrane regions with a high affinity for MC540 accounting for the inability to dissociate them. (8 Refs)

51. TRANSFORMED CELL-DERIVED INTERFERON (IFN) INDUCERS (MEETING ABSTRACT)

Smith EM, Hughes TK, Blalock JE
University of Texas Medical Branch, Galveston, TX, 77550
Fed Proc; 41(3):412 1982

Nonsensitized human and mouse lymphocyte produce IFN-alpha and IFN-alpha/beta, respectively, in response to transformed cells. Treatment of transformed human (WISH) and mouse (L) cells with trypsin, pepsin or neuraminidase resulted in a loss of the cell's ability to induce IFN-alpha. These findings suggested a transformed cell surface glycoprotein was responsible for the induction of IFN-alpha. Sonication of WISH and L cells released these glycoproteins which retained IFN inducing activity in the soluble form. The inducing molecules from WISH and L cells had molecular weights of 130,000 and 90,000 daltons, respectively. The inducer from L cells appears not to be an H-2 product. Inducer activity is not found on syngeneic and allogeneic normal cells. These results suggest the existence of a new kind of transformation antigen which may be important in lymphocyte recognition of tumor cells. (no Refs)

52. DEMONSTRATION OF TWO DISTINCT LIGHT CHAINS IN HLA-DR-ASSOCIATED ANTIGENS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS.

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Eur J Immunol; 12(3):214-221 1982

The polypeptide composition of HLA-DR-associated antigens were analyzed by two-dimensional nonequilibrium pH gradient electrophoresis (NEPHGE)/sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Glycoprotein fractions from B lymphoblastoid cell lines homozygous for the DR antigen were used as antigen source. The HLA-DR-associated antigens were isolated by immunoprecipitation using a monoclonal anti-DR antibody, TDR31.1. Two polypeptides corresponding in mol wt to the HLA-DR-associated antigen beta chain were detected. These polypeptides were shown to be different molecules on the basis of their separation on NEPHGE/SDS-PAGE analysis, their rates of turnover relative to the other polypeptides of the HLA-DR-associated antigens, and their noncoordinate alteration in position on NEPHGE/SDS-PAGE two-dimensional analysis of different cell lines. Of the two beta polypeptides, only one was invariant in position on NEPHGE/SDS-PAGE analyses of cell lines homozygous for the same DR specificity. It appears therefore that the position of one of these beta polypeptides correlates with DR specificity, while the other beta polypeptide exhibits positional variation on NEPHGE/SDS-PAGE analysis, indicative of polymorphism at a second HLA-DR-associated locus. (Author abstract) (41 Refs)

53. TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF ERYTHROCYTE MEMBRANES.

Rosenblum BB, Hanash SM, Yew N, Neel JV
Dept. Human Genetics, Univ. Michigan, 1137 E. Catherine St., Ann Arbor, MI, 48109
Clin Chem; 28(4,part2):925-931 1982

In an effort to maximize the amount of genetic information that can be extracted from a blood sample, we investigated the use of two-dimensional polyacrylamide-gel electrophoresis (PAGE) to resolve the protein constituents of the erythrocyte membrane. Lyophilized membranes were dissolve in various concentrations of urea, NP-40 detergent, and mercaptoethanol and subjected to two-dimensional PAGE by a modification of the O'Farrell procedure, with use of the ISO-DALT apparatus. More than 600 spots were visible in silver-stained gels under conditions that excluded specific cytoskeleton protein components, including spectrin and actin. The reproducibility of the pattern depended highly on the precise composition of the solubilization mixture. Poor resolution was observed in the presence of actin and other proteins of high molecular mass (spectrin bands 1 and 2) when we used high urea concentrations that solubilized the entire erythrocyte membrane. The large number of polypeptides observed could not be attributed to proteolysis, because addition of proteolytic inhibitors to the membrane wash solutions did not alter the pattern on the gel. The pattern also did not appear to include erythrocyte cytosol proteins because, except for globin, none of five purified erythrocyte lysate proteins was visible in the erythrocyte membrane gels. We conclude that two-dimensional electrophoresis provides a powerful tool for the study of non-cytoskeletal erythrocyte membrane proteins. (Author abstract) (24 Refs)

54. THE SYNTHESIS OF CELL SURFACE-ASSOCIATED ADHESIVE FACTOR BY RAT ASCITES HEPATOMA CELLS AND ITS LOCALIZATION.

Kurano R
First Dept. Pathology, Kumamoto Univ. Medical Sch., Kumamoto, Japan
Kumamoto Med J; 34(4):164-173 1981

Synthesis of a glycoprotein adhesive factor (AF) by AH136B rat hepatoma cells and its localization on the cell surface by indirect immunofluorescence is described. Binding assay of 125I-labeled antibody against AF using dissociated cultured cells demonstrated resynthesis of the AF by the cells, which was inhibited in the presence of puromycin or actinomycin D. Indirect immunofluorescence localized AF on the surface of AH136B cells, but not on AH109A cells. Immunological studies showed that the adhesive component regenerated on the surface of AH136B cells may be specifically bound with the antibody (Fab fragment) against the component, which was exogenously applied to the cells, resulting in failure of aggregation. Cell adhesion seems to require the synthesis of AF on the cell surface of AH136B cells. Separation of AF from the cells was possible for AH136B cells, but could not be accomplished with AH109A cells, suggesting that the latter do not synthesize AF. (28 Refs)

55. UTILIZATION OF GAMMA-32P-GTP BY ECTOPROTEIN KINASE OF 3T3 AND SV40 3T3 CELLS.

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Dept. Microbiology, Cell Biology, Biochemistry and Biophysics, Pennsylvania State Univ., University Park, PA, 16802
Cell Biol Int Rep; 6(4):385-392 1982

The utilization of GTP by the membrane ectoprotein kinase of 3T3 and SV40-transformed 3T3 cells (SV40 3T3) was investigated. Gamma-32P-GTP was utilized by 3T3 about 77% of that seen with ATP. The reaction was cyclic AMP and cyclic GMP-independent and required Mg ions for activity. A comparison of the phosphorylation of quiescent 3T3, serum-stimulated 3T3, and SV40 3T3 cells showed the same relative extent of phosphorylation by ATP and GTP: SV40 3T3 greater than stimulated 3T3 greater than quiescent 3T3. In each case, the amount of incorporation with GTP was less than that with ATP. Proteins from cells phosphorylated with labeled GTP or ATP were separated by polyacrylamide gel electrophoresis.

Radioautography showed generally similar labeling patterns with the two nucleotides. However, quiescent 3T3 cells exhibited a greater than 165,000 dalton protein that was labeled more extensively with ATP than with GTP. This band might represent more than one protein. The equivalent band in serum-stimulated cells was labeled to the same extent with ATP or GTP, although not as pronounced as in the quiescent cells. The rest of the banding patterns were similar to those of the quiescent cells. Thus, the 3T3 and SV40 3T3 cell ectokinase is a membrane-related to cyclic AMP-independent enzyme which can utilize GTP as well as ATP to phosphorylate similar substrates. (19 Refs)

56. A 94,000-DALTON GLYCOPROTEIN EXPRESSED BY HUMAN MELANOMA AND CARCINOMA CELLS.

Imai K, Wilson BS, Bigotti A, Natali PG, Ferrone S
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JNCI; 68(5):761-769 1982

The IgG2a monoclonal antibody (MoAb) 376.96S, secreted by a hybridoma derived from a mouse immunized with cultured human melanoma cells COLO 38, reacts with a single 94,000-dalton glycoprotein that is peripherally associated with the plasma cell membrane of cultured melanoma cells. Indirect immunofluorescence analysis with cryostat thin sections of human tissues showed that this antigen is absent from a large variety of normal tissues but is readily detectable on melanomas, nevi, and several different carcinomas. The MoAb376.96S binds with cultured melanoma and carcinoma cell lines to a similar extent and can mediate both complement-dependent and cell-dependent lysis of these cells. The 94,000-dalton glycoprotein detected by MoAb376.96S is distinct in its tissue distribution, antigenicity, and molecular profile from several structures previously identified with monoclonal antibodies that have similar molecular weights. (Author abstract) (24 Refs)

57. INFLUENCE OF THE LIVER ON THE PROFILE OF CIRCULATING ANTIGENS RECOGNIZED BY ANTISERUM AGAINST HEPATOMA MEMBRANE GLYCOPROTEINS.

Baumann H, Eldredge D
Dept. Cell and Tumor Biology, Roswell Park Memorial Inst., Buffalo, NY, 14263
Cancer Res; 42(6):2398-2406 1982

Rat hepatoma tissue culture cells have retained only a few liver-specific membrane functions. To find out whether transformation of these cells led to the appearance of tumor-specific membrane glycoprotein structures, which might also be present in released form in the circulation of tumor-bearing animals, we compared immunologically the sera and ascites fluids of normal and of hepatoma-bearing rats. For structural identification, we used polyspecific antibodies against the membrane glycoproteins of the hepatoma cells. Crossed immunoelectrophoresis of the body fluids resulted in the separation of several antigens, one of which appears to be tumor specific. Immunoelectrophorectic analyses of the glycoproteins from the membranes of liver and hepatoma cells revealed many shared antigens; in one instance, we could assign the membrane associated form to major antigen detected in the serum. Characterization of the molecular size and charge of two selected antigens by two-dimensional gel electrophoresis indicated that the hepatoma cell products were not completely sialylated. In the case of the circulating, hepatoma-specific antigen, this property appears to result in hepatic clearance of the most basic ('asialo') portion of the glycoprotein species via the receptor-mediated uptake of galactose-terminated glycoproteins. The result was the accumulation of the acidic forms of the antigen in the serum and ascites fluid of tumor-bearing rats. (Author abstract) (19 Refs)

58. AN EFFECT OF FIBRONECTIN IN EPIDERMAL GROWTH FACTOR (EGF)-STIMULATED MEMBRANE PROTEIN PHOSPHORYLATION IN HUMAN CARCINOMA CELLS.

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Centre Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Caracas 1010-A, Venezuela
Exp Cell Res; 138(2):441-446 1982

Membrane components of about 20 kd (kilodalton) which appear weakly phosphorylated in protein kinase assays of A431 human carcinoma plasma membrane to which epidermal growth factor (EGF) is added, increase their relative phosphorylation when human serum fibronectin is also included in the assay. Membranes prepared from 32PO4-labeled cells exposed to EGF and fibronectin prior to organelle isolation revealed the presence of an anodic 20 kd component not detected in cells which were not exposed to fibronectin. No comparable effects were apparent with bovine heart protein kinase and with membrane-associated protein kinase preparations which were not responsive to EGF. (Author abstract) (17 Refs)

59. PURIFICATION AND CHARACTERIZATION OF MOUSE BETA-2 MICROGLOBULIN: ALLELIC VARIANTS FROM TWO DIFFERENT STRAINS.

Ramanathan L, Dubois GC, Robinson EA, Appella E
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Mol Immunol; 19(3):435-446 1982

Beta-2-microglobulin (beta2M) is a 12,000 dalton protein associated with membrane-bound cell surface antigens. Variants of beta2M, beta2MA and beta2MB, were first detected by Michaelson et al. An improved method was used to purify beta2MA and beta2MB from BALB/c and C57BL/6 mouse livers, respectively. Reproducible yields of 10% were obtained. The purifications were accomplished by a 3 M sodium thiocyanate (NaSCN) extraction of a crude membrane fraction, an acid precipitation step, gel filtration on Sephadex G-75 and ion-exchange of a crude membrane fraction, an acid precipitation step, gel filtration on Sephadex G-75 and ion-exchange chromatography on DEAE-cellulose and CM-cellulose in that order. The elution profile of beta2MA and beta2MB on the ion-exchange columns was found to be different, indicating the presence of structural changes. Beta2MA was found to be more acidic (pI = 7.35) than beta2MB (pI = 7.68) by isoelectric focusing in gels. Complete sequence analysis of beta2MA and partial sequence analysis of beta2MB (61 of 99 residues) were performed by automated Edman degradation of the intact chain and of the overlapping peptides obtained by: (a) tryptic cleavage at arginines after acetimidation of lysine side chains, (b) BNPS-skatole cleavage at trytophan residues and (c) hydroxylamine cleavage at asparagine-glycine linkages. A comparison of the primary structure of beta2MA to the partial amino acid sequence obtained for beta2MB revealed a single amino acid substitution (aspartic acid for alanine at position 85) that accounts for the differences in biochemical properties observed. (Author abstract) (46 Refs)

60. SOLUBILIZATION OF AN ACTIVITY REGULATING C3B FUNCTION FROM RAJI CELL MEMBRANES.

Carlson P, Ruddy S, Conrad DH
Dept. Medicine and Microbiology, Medical Coll. Virginia, Richmond, VA, 23298
Immunology; 46(1):163-173 1982

A fraction of isolated Raji cell membranes solubilized with 2 M KBr which was capable of inhibiting C3-dependent rosettes was examined for its ability to inhibit the alternative pathway of complement. It was shown to decrease alternative pathway-dependent hemolysis of sheep erythrocytes and to accelerate decay of factor B from these cells. It had no effect on C2 decay, the classical pathway analog of factor B. Inhibitory activity solubilized from Raji cells was not removed by immunoadsorption with anti-factor H or anti-factor I, two well-characterized serum C3b-control proteins. It also differed in two functional respects from these proteins. Firstly, it failed to result in cleavage of a peptide bond in C3 which is characteristic of factor I; secondly, its inhibitory activity did not synergize with factor I in inhibiting the alternative pathway, unlike factor H. These results suggest that Raji cells contain a regulatory factor in their membranes for the alternative pathway of complement which is distinct from factors H and I. (Author abstract) (18 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

61. DIFFERENTIAL PHOSPHORYLATION OF SOLUBLE VERSUS RIBOSOME-BOUND EUKARYOTIC INITIATION FACTOR 2 IN THE EHRLICH ASCITES TUMOR CELL.

Wong ST, Mastropaolo W, Henshaw EC
Univ. Rochester, Cancer Center, Rochester, NY, 14642
J Biol Chem; 257(9):5231-5238 1982

The Ehrlich cell contains eukaryotic initiation factor 2 (eIF-2) kinase(s) and eIF-2 phosphatase(s), and a regulatory role for eIF-2 phosphorylation in protein synthesis has been postulated. In order to assess the extent of phosphorylation of the alpha subunit of eIF-2 in the Ehrlich cell, eIF-2 was partially purified from cell fractions by phosphocellulose chromatography and was examined by isoelectric focusing, which resolves the phosphorylated and unphosphorylated forms. Post-mitochondrial supernatant preparations of exponentially growing cells were divided into a soluble and a sedimentable fraction by centrifugation at 166,000 x g for 2 1/2 hr, and eIF-2 in each fraction was analyzed. In the absence of precautions to inhibit phosphatase, less than 10% of eIF-2 was phosphorylated in either fraction. However, when all procedures were carried out in the presence of the phosphatase inhibitor, 50 mM NaF, eIF-2 in the soluble fraction was greater than 90% phosphorylated while eIF-2 in the sedimentable fraction was only 40% phosphorylated. Phosphorylated or unphosphorylated eIF-2 which was added to the NaF-containing cell homogenate before fractionation showed no gain or loss of phosphorylation during processing. Thus, the difference in phosphorylation between soluble and sedimentable eIF-2 was not a result of sample processing. Since the sedimentable eIF-2 contains all the eIF-2 which is ribosome-bound, and therefore is in the process of functioning, our findings support the hypothesis that, in the Ehrlich cell, phosphorylated eIF-2 is less likely to form initiation complexes and function in protein synthesis than unphosphorylated eIF-2. (Author abstract) (59 Refs)

62. ALPHA-1-ACID GLYCOPROTEIN: MICROHETEROGENEITY AND SYNTHESIS BY HUMAN ADENOCARCINOMA (MEETING ABSTRACT)

Tokes ZA, Gendler SJ, Pullano TG, Ross KL
University of Southern California, Los Angeles, CA, 90033
Fed Proc; 41(3):1158 1982

Glycoproteins synthesized and released by short-term cultures of fresh surgical specimens from breast, prostate and lung adenocarcinoma patients were analyzed. 14C-glucosamine was incorporated into nondialyzable macromolecules which were characterized using 2D gel electrophoresis and immunoprecipitation. The incorporation was tunicamycin sensitive. Five to seven families of glycoproteins were synthesized by the organ cultures from all three sites. One of the families was identified by immunoprecipitation as alpha-1-acid glycoprotein (AGP) which comprised one to six percent of the total of labeled components. Both the tumor-and the normal serum-derived AGP can be resolved into at least seven major subgroups using high resolution 2D gel electrophoresis. Disproportionality of AGP subgroups from adenocarcinoma specimens and normal sera was observed. Surgical removal of the tumor resulted in a change in the AGP subgroup distribution which indicates that at least a portion of the previously reported elevations of AGP in the sera of cancer patients may represent actual tumor product. Therefore, the relative proportions of AGP subgroups in the serum have diagnostic significance. Since AGP is a known inhibitor of lymphoblastogenesis, the expression of this glycoprotein by the tumor cells may have prognostic significance as well. (no Refs)

63. ONE OR PERPHAPS TWO GENES DIRECT THE SYNTHESIS OF THYMUS-LEUKEMIA ANTIGENS (MEETING ABSTRACT)

Cohen EP, Slomski R, Martens C
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Fed Proc; 41(3):414 1982

Thymus-leukemia (TL) antigens consist of a light chain of 12,000 daltons and a glycosylated heavy chain of 47,000 daltons forming TL antigenic determinants. We obtained mRNA (messenger RNA) specifying the heavy chain of TL antigens by isolating poly(A)-containing RNA from polysomes of ASL-1 cells, a TL(+) mouse leukemia cell line. Polysomes forming TL antigens were immunoprecipitated with antiserum specific for TL 1,2,3 antigenic determinants. The mRNA obtained directed the synthesis in a reticulocyte lysate system of protein of 42,000 daltons, the non-glycosylated heavy chain. 32P-labeled cDNA was prepared from the mRNA. DNA from ASL-1 cells was digested with EcoRI, Bam I or Hind III restriction endonucleases. Using the Southern 'blot' technique, cDNA for TL antigens hybridizes with two separate DNA segments in each restriction digest. Using solution hybridization methods, cDNA for TL antigens hybridizes with mouse DNA according to single copy kinetics. The experimental approaches used reveal the presence of one, or perhaps two genes for this integral membrane protein. (no Refs)

64. BIOSYNTHESIS OF RAT INSULINS I AND II: EVIDENCE FOR DIFFERENTIAL EXPRESSION OF THE TWO GENES.

Kakita K, Giddings S, Permutt MA
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Proc Natl Acad Sci USA; 79(9):2803-2807 1982

The purpose of these experiments was to determine the relative content and biosynthetic rate of insulins I and II under various experimental conditions. The two insulins were quantitated by polyacrylamide gel electrophoresis, electrotransfer to nitrocellulose paper, photoaffinity crosslinking, and immunodetection with anti-insulin antibody and 125I-labeled protein A. The ratio (mean +- SEM) of insulins, I/II, was 1.2 +-0.2 in Wistar-Furth rats fasted for 4 days, 1.6 +-0.2 in normal rats, and 5.5 +-0.8 in growth hormone-tumor-bearing hyperinsulinemic rats (p less than 0.01). The increase in content of rat insulin I compared to II in the growth hormone-tumor-bearing animals was confirmed by radioimmunoassay of gel slices. To determine whether the differences in contents of rat insulins I and II in the hyperinsulinemic rats was due to increased biosynthesis or a different turnover rate, isolated rat islets were incubated in [3H]leucine for 4 hr with 5.5 mM or 16.0 mM glucose in the incubation medium. Glucose stimulated insulin biosynthesis greater than eight-fold. The ratio of synthesis of rat insulin I relative to II was 0.9 +-0.1 at 5.5 mM glucose and 9.8 +-3.3 (p less than 0.01) at 16.0 mM glucose. Therefore, under conditions that stimulate insulin biosynthesis, there was a marked preferential synthesis of rat insulin I relative to II. These studies suggest that the two rat insulin genes are expressed independently and that, under stimulatory conditions, there is preferential expression of the rat insulin I gene. (Author abstract) (35 Refs)

65. S-100 PROTEIN SYNTHESIS IN CULTURED GLIOMA CELL IS G1-PHASE OF CELL CYCLE DEPENDENT.

Fan K
Dept. Pathology, Veterans Admin. Medical Center, Little Rock, AR, 72206
Brain Res; 237(2):498-503 1982

S-100, a brain specific acidic protein predominantly glial in origin, is synthesized in a differentiated glioma cell line (C6), adapted from a chemically-induced brain tumor in rat. When G1 phase synchronized C6 cells were cultured in sparse conditions, the S-100 protein synthesis rate did not differ from confluent cultures with cell to cell contact; confluency apparently was not an absolute requirement. S-100 protein seems dependent upon the cells entering the stationary intermitotic phase, specifically the G1 phase or the hypothetical G0 phase. (24 Refs)

66. SYNTHESIS AND DEGRADATION OF HEAT SHOCK PROTEINS DURING DEVELOPMENT AND DECAY OF THERMOTOLERANCE.

Landry J, Bernier D, Chretien P, Nicole LM, Tanguay RM, Marceau N
Centre de Recherche sur le Cancer de l'Universite Laval, L'Hotel-Dieu de Qubec, Quebec G1R 2J6, Canada
Cancer Res; 42(6):2457-2461 1982

Morris hepatoma 7777 cells, heat conditioned at 43 C for 0.5 hr, become gradually thermoresistant during an incubation at 37 C as judged by their ability to form colonies following a second heat challenge. Pulse incorporation of [35S]methionine into proteins at various times after the conditioning treatment and subsequent fractionation of the proteins by polyacrylamide gel electrophoresis indicate that the gradual putative modifications occurring at the cellular level and leading to the thermotolerance state are accompanied by an elevated synthesis above the normal level of a small set of polypeptides with apparent molecular weights of 27,000, 65,000, 68,000, 70,000, 89,000, and 107,000. Both thermotolerance development and protein induction are completed after a 6-to 8-hr period. At the end of this period, thermotolerance is at its maximum level and heat shock protein synthesis is returned to normal. This acquired thermal resistance eventually disappears between 60 and 80 hr following conditioning treatment. In a parallel manner, the heat shock-induced proteins synthesized during the first 4 hr following the conditioning treatment are maintained in the cells at a high level for several hr but become undetectable by 82 hr. The results provide strong circumstantial evidence that heat shock proteins are involved in the acquisition, maintenance, and decay of thermotolerance. (Author abstract) (40 Refs)

67. REUTILIZATION OF AMINO ACID CARBONS IN RELATION TO ALBUMIN TURNOVER IN NONGROWING MICE WITH SARCOMA.

Karlberg I, Ekman L, Edstrom S, Schersten T, Lundholm K
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Cancer Res; 42(6):2284-2288 1982

Reutilization of amino acid carbons was evaluated in relation to increased turnover of albumin in tumor-bearing mice. A methylcholanthrene-induced sarcoma (MCG 101) was used in nongrowing mice (C57BL/6J). Sarcoma-bearing mice developed hypoalbuminemia, but pair-fed controls did not. The hypoalbuminemia was caused by increased albumin degradation rate, measured by injection of Na214CO3, and by exponentially increased deposition of albumin into the tumor compartment. The fractional synthesis rate of albumin was doubled in tumor-bearing mice compared with controls. The translational capacity of albumin synthesis evaluated in vitro was maintained in tumor host livers. The recycling of [14C]leucine carbons was almost extinguished in plasma albumin of sarcoma-bearing mice, while that of control mice contributed to 30 to 40% of the total leucine carbon flux in turned over albumin. The recycling of arginine carbons was also different when measured after simultaneous injection of [guanido-14C]arginine and [2,3-3H]arginine. The hepatic pool of free leucine was increased by 22% in tumor-bearing mice. It is concluded that increased albumin degradation in cancer may be a disordered event and is earlier and of initially greater quantitative importance than is altered synthesis of albumin for the development of hypoalbuminemia in experimental cancer. (Author abstract) (34 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

68. IDENTIFICATION OF A PHOSPHOPROTEIN SPECIFICALLY INDUCED BY THE TRANSFORMING DNA OF RAT NEUROBLASTOMAS.

Padhy LC, Shih C, Cowing D, Finkelstein R, Weinberg RA
Center Cancer Res., Cambridge, MA, 02139
Cell; 28(4):865-871 1982

DNAs from nitrosoethylurea-induced rat neuroblastomas transform NIH/3T3 mouse fibroblasts in a transfection assay. DNAs of such transformed cells can be used in a subsequent cycle of transfection to generate secondary foci that contain virtually no foreign genetic material besides the sequences carrying the rat neuroblastoma transforming function. These secondary neuroblastoma transfectants were injected into young mice and grew out into fibrosarcomas. Sera from these mice were examined for reactivity with any proteins which were induced specifically by the neuroblastoma transforming sequence. These sera precipitate a polypeptide of about 185,000 daltons from 35S-methionine-labeled cell lysates of the rat neuroblastoma cells that served as DNA donors and in all transfection-drived primary and secondary foci. This protein is present in high levels in all neuroblastoma transfectant clones, but was not detectable in a variety of other transformed cells. Antisera were prepared from mice bearing tumors induced by transformed cells derived by transfection of DNAs from various tumor cell types unrelated to rat neuroblastoma. These antisera failed to immunoprecipitate the 185,000 dalton protein. These data indicate that the synthesis of the 185,000 dalton protein is specifically induced by the neuroblastoma transforming sequence. The protein may be encoded by the transforming sequence and may mediate transformation in this chemically induced tumor. (Author abstract) (9 Refs)

69. SYNTHESIS AND TURNOVER OF REGULATORY SUBUNIT OF PROTEIN KINASE I IN NEUROBLASTOMA CELLS (MEETING ABSTRACT)

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Fed Proc; 41(3):659 1982

Multiple forms of regulatory subunit of PKI (R1 and R) exist in mouse neuroblastoma cells. R1 and R have a MW of 48,000; however, the pI are different, R1 (pI 5.2) and R (pI 5.4). DibutyrylcAMP increased R by 3-fold without affecting R1. Pulse labeling of cells with 35(s)met for 5 min, show an increase in R by 2-fold in dibutyrylcAMP treated cells compared to control cells, suggesting increase in R is due to de novo synthesis. Translation of 1ug of poly(A)+RNA in reticsys show a 2-fold increase in the synthesis of R by poly(A)+RNA from dibutyrylcAMP treated cells compared to control cells. These results indicate dibutyrylcAMP-induced R is due to increase in the specific mRNA, suggesting different mechanisms for the synthesis of R1 and R. The turnover rates of R1 and R were determined by chasing 35(S)met labeled cells in the absence and presence of dibutyrylcAMP. The half-life of R in the absence of dibutyrylcAMP was 25 hr and in the presence of dibutyrylcAMP R was stabilized, decreasing 16% after 48 hr. In contrast, R1 was stable in the absence or presence of dibutyrylcAMP, decreased by 23% after 48 hr. These data show R1 and R have different turnover rates. Therefore, dibutyrylcAMP induced R, due to increased synthesis as well as increased stability due to R-cAMP complex formation. Characterization and understanding the functions of multiple forms of regulatory subunit will help to understand their role in cellular functions, especially in dibutyrylcAMP-induced differentiation. (no Refs)

70. EFFECTS OF PCB ON THE SYNTHESIS AND THE DEGRADATION OF PROTEINS COMPOSING MICROSOMAL MEMBRANES OF RAT LIVER.

Narbonne JF
Laboratoire de Toxicologie alimentaire, Avenue des Facultes, 33405 Talence, France
Trav Soc Pharm Montpellier; 41(1/2):141-142 1981

Synthesis and turnover of total proteins and isolated proteins (cytochrome B5 and cytochrome P450) in membranes of hepatic endoplasmic reticulum were studied in rats being fed polychlorobiphenyls (PCB). Rats receiving a regimen containing 20-100 ppm PCB showed a large increase (2-6x) in protein synthesis. Rats receiving low doses of PCB showed no change in the speed of protein degradation. (2 Refs) (In FRE)

71. EFFECT OF BACTERIALLY PRODUCTED INTERFERON-ALPHA2 ON SYNTHESIS OF SPECIFIC PEPTIDES IN HUMAN PERIPHERAL LYMPHOCYTES.

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FEBS Lett; 140(1):109-112 1982

I-peptide enhancement obtained in human lymphocytes using virtually pure human interferon alpha2 (INF-2), produced by Escherichia coli after genetic manipulation is described. INF-2 was capable of enhancing the synthesis of the same eight peptides in human T-cells as conventionally produced INF. However, the bacterially-produced INF-2 did not produce this effect in all donors, suggesting a limited range of donor or cell-type specificities. (13 Refs)

72. THE RELATIONSHIP BETWEEN THE INSULIN CONTENT AND INHIBITORY EFFECTS OF BOVINE COLOSTRUM ON PROTEIN BREAKDOWN IN CULTURED CELLS.

Ballard FJ, Nield MK, Francis GI, Dahlenburg GW, Wallace JC
CSIRO Div. Human Nutrition, Adelaide SA 5000, Australia
J Cell Physiol; 110(3):249-254 1982

The sensitivity of protein breakdown in several cell lines to inhibition by bovine colostrum was compared. In the presence of 2% colostrum the largest percentage inhibition was observed with Swiss 3T3 cells (35%) and the smallest with T47D breast cells (16%). All 10 lines tested showed marked inhibitory effects when 0.5% colostrum was added, giving a response approximately equivalent to that observed with the addition of 10% fetal calf serum. Unpasteurized milk, tested at 2% against each cell line, produced effects approx equal to that found with 0.1% colostrum. The wide range of cell types studied was chosen to provide bioassays for different growth factors, including insulin, epidermal growth factor, insulin-like growth factors, and fibroblast growth factor. The two hepatoma cell lines studied (H35 and MH1C1) were observed to be atypically sensitive toward insulin, and inhibition of protein breakdown in these cells was proportional to the insulin content of colostrum to which they were exposed. Insulin was not responsible for the inhibition of protein breakdown observed in other cell lines. The factors responsible remain unknown. Growth factors other than insulin were suggested as likely candidates. (25 Refs)

73. REGULATION OF CATABOLISM OF IGM HEAVY CHAINS IN A B LYMPHOMA CELL LINE.

Dulis BH, Kloppel TM, Grey HM, Kubo RT
Dept. Medicine, Natl. Jewish Hosp. and Res. Center, Denver, CO, 80206
J Biol Chem; 257(8):4369-4374 1982

The human lymphoma cell line Daudi has the phenotype of a nonsecreting B cell. This cell line synthesizes both the membrane and secreted forms of the IgM heavy chain, but only expresses functional membrane IgM. We have found that secreted type heavy chains (mu s) are rapidly degraded in these cells, with a half-life of 1.3 h. Some of the membrane type heavy chains (mu m) are also rapidly catabolized but some are expressed in a stable form with a half-life of 13 h. Inhibiting the initial glycosylation of heavy chains with tunicamycin has differential effects on the catabolic rates of mu s and mu m chains. The turnover of mu s chains is not affected by this inhibitor, but the degradation of mu m chains is much more rapid after tunicamycin treatment. In comparison with their glycosylated counterparts, nonglycosylated mu chains do not covalently assemble to a significant degree with light chains. Tunicamycin treatment of Daudi cells thus seems to inhibit formation of stable mu m protein, possibly by altering mu chain conformation and inhibiting its interaction with light chains. We conclude from these results that some mu m chains are specifically protected from proteolysis by posttranslational events. These processing events include covalent assembly with light chains, terminal glycosylation, and insertion into the plasma membrane. (Author abstract) (25 Refs)

74. INHIBITION OF BLEOMYCIN-INDUCED PULMONARY FIBROSIS BY COBRA VENOM FACTOR.

Phan SH, Thrall RS
Dept. Pathology, Univ. Michigan Medical Sch., Box 045, Ann Arbor, MI, 48109
Am J Pathol; 107(1):25-28 1982

A single dose of cobra venom factor, a known, potent depletor of serum complement, has recently been reported to be highly effective in inhibiting lung collagen deposition at 7 days after bleomycin treatment. The present study shows that this is associated with suppression of the bleomycin-induced increase in collagen synthesis, down to virtually normal levels. There is also a concomitant reduction in tissue free proline pool size. No significant effects are noted in noncollagenous protein synthetic rates. This inhibitory effect is not seen at 30 days after bleomycin and is correlated with a return to normal levels of serum complement hemolytic activity. These data suggest an important role for complement activity in the lung's fibrotic response to bleomycin. (Author abstract) (20 Refs)

75. INFLUENCE OF 3-METHYLCHOLANTHRENE ON LIVER NUCLEOLAR AND NUCLEOPLASMIC ACTIVITIES OF PROTEIN KINASES AND RNA POLYMERASES.

Kleeberg U, Szeberenyi J, Juhasz P, Tigyi A, Klinger W
Inst. Pharmacology and Toxicology, Friedrich-Schiller Univ., Jena, E. Germany
Biochem Pharmacol; 31(6):1063-1067 1982

The experiments were designed to investigate some details of the action of 3-methylcholanthrene (3-MC) on the regulation of transcription. After a single intraperitoneal dose of 3-MC a significant increase in the activities of both nucleolar and nucleoplasmic protein kinases in hepatic cells of young rats was found. The maximal stimulation took place 24 hr after the administration of 3-MC and the extent of activation was much greater in the nucleolar fraction. There is a significant elevation of the activities of both functional forms, free and template-engaged, of RNA polymerase A 24 hr after a single injection of 3-MC. Free and engaged forms of extranucleolar RNA polymerase B show a different behavior: after 24 hr of 3-MC administration the engaged form is markedly enhanced while the activity of the free enzyme shows a significant decrease. The more moderate increase in total RNA polymerase B activity is obviously preceded by a transfer of the enzyme from 'free' to 'engaged' form. Since the enhancement of protein kinase activities was accompanied by the stimulation of nuclear RNA polymerases we suggest that both kinds of enzymes are involved in an epigenetic mechanism of the inducing action of 3-MC on cytochrome P1-450. (Author abstract) (47 Refs)

76. CHLORAMBUCIL-INDUCED CHANGES IN CHROMATIN METHYLATION (MEETING ABSTRACT)

Ramirez ML, Shepherd R, Pinsky S, McGhee K, Harrap KR
Department of Biochemical Pharmacology, Institute of Cancer Research, Sutton, Surrey, England
Br J Cancer; 44(2):304 1981

Post-synthetic methylation of nucleic acids and nuclear proteins is a putative regulatory event in the function and structure of chromatin. Changes in methylation may also be determinants of alkylating-agent toxicity, in the same way as has been shown for chromatin protein phosphorylation. We studied the effects of chlorambucil treatment on DNA and nuclear protein methylation in sensitive (S) and resistant (R) Walker 256 carcinosarcoma cells in vitro (2 h treatment) and in vivo (24 h treatment). Incorporation of [3H-methyl]-methionine (Met) into whole cells or [3H-methyl] S-adenosylmethionine (SAM) into isolated nuclei was measured. At an ID50 dose, DNA, histone and non-histone methylation was stimulated in S cells but not in R cells (Met assay). An ID90 dose inhibited methylation in both S and R cells. Similar results were obtained using isolated nuclei, were only histone methylation was detected (non-histone methyl-transferases are cytoplasmic enzymes). DNA methylation at the 5 position of cytosine was inhibited at ID90 doses. The transcriptional inducer, sodium butyrate, at an ID90 dose in vitro (5 mmol/l for 48 h) inhibited methylation by 50-70%, while an ID10 dose (2 mmol/l, 24 h) caused less inhibition. Pretreatment with butyrate inhibited chlorambucil-mediated enhancement of methylation. These data indicate that changes in methylation of chromatin proteins and DNA paralleled alkylating-agent toxicity. Nuclear protein methylation has been associated with chromatin condensation, and its inhibition at ID90 doses correlated with the loss of heterochromatin observed in these cells. (no Refs)

DIETARY AND NUTRITIONAL EFFECTS

77. ESSENTIAL AMINO ACID CONTROL OF ALBUMIN SYNTHESIS IN HEPATOMA CELLS (MEETING ABSTRACT)

Ledford BE, Davis DF
Department of Biochemistry, Medical University of South Carolina, Charleston, SC, 29425
Fed Proc; 41(3):1168 1982

The synthesis of albumin in the liver has been shown to correlate with the availability of essential amino acids in the diet. The cell line Hepa is a highly differentiated mouse hepatoma which secretes serum proteins. These cells provide a well defined experimental system for further investigating the role of essential amino acids in the control of albumin synthesis. We have chosen to study lecine, isoleucine, phenylalanine, histidine and tryptophan based on their abundance in mouse albumin. Hepa cells were grown for three days in complete medium (DME, 2% fetal calf serum (FCS)) with daily medium changes. The cells were then incubated for 22 hr in media containing varying concentrations of the individual essential amino acids supplemented with 2% dialyzed FCS. The deficient media were then changed and 1.5 hr later the cells were labeled for 0.5 hr with 3H-leucine. Albumin was immunoprecipitated and total protein was TCA (trichloroacetic acid) precipitated from postribosomal supernatants of detergent, solubilized cells. Incorporation of leucine into albumin was 4.3% of total incorporation in complete medium. With the exception of isoleucine, the relative rate of albumin synthesis decreased as a function of amino acid concentration of 2.5% in totally deficient media. The specificities of these amino acid effects on the synthesis of albumin were further investigated by quantitation of fluorograms sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels run on the postribosomal supernatants. The major change in the patterns was a decrease in the labeling of albumin. Thus tryptophan, leucine, histidine, and phenylalanine depletion regulate the relative rate of albumin synthesis with a high degree of specificity. (no Refs)

78. EFFECT OF DIETARY PROTEIN ON TUMOR GROWTH AND PROTEIN STATUS OF C3H MICE (MEETING ABSTRACT)

Kamath SK, Conrad NC, Kohrs MB, Ghosh L
Department of Nutrition and Medical Dietetics, University of Illinois, Chicago, IL, 60612
Fed Proc; 41(3):355 1982

The effect of dietary protein on tumor growth and protein status of C3H mice with mammary adenocarcinoma was investigated in a two part study. In part 1, animals with (T) or without (0) tumor fed ad lib for 21 days, a chow diet (A) or a 20% (B) or a 15% casein diet (C) were compared. In part 2, diets A, B, C, and also those containing 15% amino acid (aa) mixture (D), 15% aa with tryptophan (trp) content reduced to 25% of diet D (E), 15% zein supplemented with lysine and trp (F) and 15% zein supplemented with lysine and trp (F) the latter reduced to 25% of diet F (G) were tested in T animals. The T animals had consistently lower levels of hematocrit and serum albumin and increased liver weight and liver nitrogen compared to the 0 counterparts. Regardless of diet or disease, all animals were in positive nitrogen balance. The kind or amount of protein had no effect on serum albumin concentration or tumor growth, the latter judged by its weight, volume, change in volume and nitrogen content. However, mean values for tumor free body weight, creatinine, liver weight, liver nitrogen, hematocrit and nitrogen retention tended to be higher in groups TA and TB, thus indicating that an increased protein intake may be beneficial to the host without aggravating the tumor. (no Refs)

79. HYPERCALORIC NUTRITION OF PATIENTS WITH CANCER OF THE DIGESTIVE TRACT.

Cuocolo R, De Martino G, Ortolani O
Istituto di Anestesiologia e Rianimazione, II Facolta di Medicina e Chirurgia, Universita degli Studi di Napoli, Napoli, Italy
Minerva Chir; 36(23/24):1601-1610 1981

Sources of energy in men were reviewed and schematically presented during fast and after trauma. Protein status before and after surgery was analyzed. Of 20 patients (9 men, 11 women), 5 had gastric cancer and underwent radical surgery, 15 had colon cancer, 11 underwent radical and 4 palliative surgery. A wk before surgery increasing amounts of glucose (10-33%) solutions with steady amounts of amino acids (8.5%) and other nutrients were administered. First day after surgery the glucose amount was decreased, but the following days nutrition was the same as before the surgery. Parenteral nutrition (PN) was stopped 10 days after surgery, and the amount of glucose was decreased gradually. Nitrogen balance (NB), azotemia, glycemia, glycosuria, Na, and K were determined daily. Different proteins were determined by electrophoresis twice weekly, and many other tests were performed once or twice weekly. Protein values increased before surgery, decreased after surgery, but were higher than at the start of the PN, and increased again at the end of the PN. The PN did not completely abolish the effect of surgery, but it did decrease its effect. (20 Refs) (In ITA)

POLYAMINES - SYNTHESIS AND METABOLISM

80. ORNITHINE DECARBOXYLASE ACTIVITY IN CHEMICALLY INDUCED MOUSE SKIN PAPILLOMAS.

Astrup EG, Boutwell RK
Inst. Pathology, Univ. Oslo, Rikshospitalet, Oslo 1, Norway
Carcinogenesis; 3(3):303-308 1982

The activity levels of L-ornithine carboxy-lyase (ODC) (EC 4.1.1.17) and S-adenosyl-L-methionine carboxy-lyase (SAM-D) (EC 4.1.1.50) were determined in individual papillomas induced in mouse skin by a two-stage technique, and in normal mouse epidermis. Cycloheximide treatment abolished both enzyme activities. In normal epidermis the ODC activity was barely detectable, whereas the tumors exhibited high levels of ODC. Levels of SAM-D activity above those of normal epidermis were detected in some papillomas, but in contrast to ODC the SAM-D activity levels were not consistently increased in skin tumors. By pooling a great number of papillomas, the variations in ODC and SAM-D activities between different papillomas could be minimized so that reliable measurements of the biological half-lives of ODC and SAM-D in the tumors were obtained using cycloheximide treatment. The half-life of SAM-D in squamous papillomas was 45 min, almost identical to the 41 min half-life of the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced level of this enzyme in normal mouse epidermis. In contrast, the ODC activity of the mouse skin papillomas declined at a rate similar to that in TPA-treated epidermis for only the first 15-20 min after cycloheximide injection. Thereafter, at time points when protein synthesis was approx 90% inhibited, the ODC activity reverted to high levels. These results show that the high level of ODC activity in squamous papillomas is stabilized. This observation is compatible with the hypothesis that the control mechanism of the ODC activity level in these tumors is severely deranged. This change in polyamine turnover pattern may be related to altered differentiation of the epidermal cells, which constitute the main bulk of cells in these tumors. (Author abstract) (40 Refs)

81. CHANGES OF ORNITHINE DECARBOXYLASE ACTIVITY AND POLYAMINE CONTENT UPON DIFFERENTIATION OF MOUSE NB-15 NEUROBLASTOMA CELLS.

Chen KY, Presepe V, Parken N, Liu AY
Dept. Chemistry, Rutgers-The State Univ. New Jersey, New Brunswick, NJ, 08903
J Cell Physiol; 110(3):285-290 1982

Specific changes of basal level of ornithine decarboxylase (ODC) activity and spermidine content during the differentiation of murine NB-15 neuroblastoma cells and the temporal relationship of these changes and the expression of differentiated phenotypes of neuroblastoma cells are reported. ODC activity of nondifferentiating (NB) neuroblastoma cells and differentiating (ND) cells was studied over a 5 day period. NB cells showed an early increase in ODC activity that was temporarily associated with the resumption of logarithmic growth after plating. Upon entering the stationary phase of cell growth, ODC activity in NB cells decreased precipitously, reaching a minimum activity of 0.2 units/mg protein at the end of the 5 day culture period. In ND cells, the early increase in ODC activity was attenuated in both magnitude and duration. The increase in ODC activity of ND cells was transient. While putrescine levels were similar in NB and ND cells. Significant differences were observed in levels of cellular spermidine. Spermidine content of NB cells followed a steady increase from the basal level of 1.5 nanoMols (nM)/mg protein to a maximal value of 5.05 nM/mg protein. This elevated level of spermidine in NB cells was sustained for the entire 5 day period. The significance of cAMP-induced changes in ODC activity and polyamine contents in cell differentiation are discussed. (15 Refs)

82. AN ORNITHINE DECARBOXYLASE-DEFICIENT MUTANT OF CHINESE HAMSTER OVARY CELLS.

Steglich C, Scheffler IE
Dept. Biology, B-022, Univ. California, San Diego, La Jolla, CA, 92093
J Biol Chem: 257(8):4603-4609 1982

We have selected a mutant of Chinese hamster ovary cells that is severely deficient in ornithine decarboxylase activity and is auxotrophic for putrescine. The mutant we obtained (C54) has only 3% of the max inducible ornithine decarboxylase activity of the parental Chinese hamster ovary cells and the rate of incorporation of [3H]ornithine into acid-soluble material is correspondingly reduced. The defect in the mutant was recessive in somatic cell hybrids. The mutant requires at least 10(-5) M putrescine in the medium to maintain a normal growth rate. Spermidine and spermine can also serve as a polyamine source, and very high (millimolar) concentrations of ornithine can support a normal growth rate. In the absence of polyamine supplementation the cells stop growing after about three population doublings and begin to die after 5 or 6 days. The intracellular concentrations of putrescine and spermidine are depleted after 24 and 48 hr, respectively. Spermine levels remain essentially constant for 4 days. Unexpectedly, the Km for ornithine of the enzyme from mutant cells was consistently somewhat lower than the Km for the enzyme from wild type cells. It is possible but not yet certain that the mutant is the result of a mutation in the structural gene coding for ornithine decarboxylase. (Author abstract) (52 Refs)

83. INHIBITION OF ORNITHINE DECARBOXYLASE AND S-ADENOSYLMETHIONINE DECARBOXYLASE ACTIVITIES OF S49 LYMPHOMA CELLS BY AGENTS INCREASING CYCLIC AMP.

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J Cycl Nucleotide Res; 7(5):321-332 1981

Regulation of two key enzymes which control polyamine biosynthesis, L-ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC), by agents increasing cyclic AMP (cAMP) was studied in various S49 lymphoma cell lines. Incubation of wild type S49 cells with beta-adrenergic agonists (terbutaline or isoproterenol) led to more rapid inhibition (less than 2 hr) of activities of both enzymes than cells in the G1 phase. Propanolol (beta-adrenergic antagonist) blocked inhibition of ODC by isoproterenol but only if added simultaneously or less than 4 hr after the agonist. Decreases in ODC activity produced by the beta-adrenergic agonists, cholera toxin, prostaglandin E1 or dibutyryl cAMP were all enhanced by the phosphodiesterase inhibitor Ra 20-1724. Results of studies of ODC and SAMDC activity in S49 variants having lesions in the pathway of cAMP generation and action indicate that the 'classical' components of the hormone receptor-cAMP-protein kinase pathway are involved. These results in S49 cells contrast with evidence in other systems in which cAMP has been suggested to enhance polyamine biosynthesis, perhaps through alternative mechanisms. (36 Refs)

84. UPTAKE INTRACELLULAR DISTRIBUTION AND METABOLISM OF N-N1-DIACETYL-14C-PUTRESCINE (DDAB), A POTENT INDUCER OF FRIEND ERYTHROLEUKEMIA DIFFERENTIATION (MEETING ABSTRACT)

Canellakis ZN, Bondy PK, Gazitt Y
Veterans Administration Medical Center, West Haven, CT
Fed Proc; 41(3):682 1982

Previous publications have implicated ornithine decarboxylase and the polyamines in the induction of differentiation in Friend erythroleukemia (FL) cells. We now report the uptake and metabolism of DDAB in FL cells induced to differentiate by 10mM DDAB (1uCi/umole). The results indicate a gradual increase during induction in the amount of DDAB taken up by 5x10(7) cells within 24 hours to the cytosol (14.4 n moles), nuclear fraction (5.8 n moles) and membrane fraction (15.8 n moles). Concurrently, there is an increase in the extent of conversion of DDAB into the major metabolites, putrescine, spermidine and spermine. At 24 hours 40% of cytosolic DDAB is metabolized and appears as putrescine (11%), spermidine (15.4%) and spermine (2%). We also detected small amounts of N-Monoacetyl putrescine (2%) and N-acetyl spermidine (1%). (no Refs)

85. ROLE OF PHOSPHOLIPASE A2 AND PROSTAGLANDIN E IN GROWTH AND DIFFERENTIATION OF MYELOID LEUKEMIC CELLS.

Simantov R, Sachs L
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Biochim Biophys Acta; 720(2):111-119 1982

Phospholipase A2 activity and prostaglandin E synthesis have been studied in different clones of myeloid leukemic cells, which differ in their competence to be induced to differentiate by the macrophage and granulocyte differentiation-inducing protein or the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA). Clones that could be induced to differentiate by this protein showed a higher basal phospholipase A2 activity than clones that could not be induced to differentiate by this protein inducer. Cell competence to be induced to differentiate by TPA did not show this correlation, and the clone with the least ability to respond to TPA showed the lowest number of binding sites for [20-3H]phorbol 12,13-dibutyrate. Differentiation induced by the protein was accompanied by a 7-14-fold increase in prostaglandin E synthesis, whereas differentiation induced by TPA did not show this increase. Externally added prostaglandin E1 did not induce differentiation but inhibited cell proliferation and the degree of inhibition in the different clones was related to the basal phospholipase A2 activity. The results indicate that increase of prostaglandin E synthesis was not an essential pre-requisite for differentiation, that prostaglandin E seems to be involved in the inhibition of cell proliferation in association with phospholipase A2, and that the differentiation-inducing protein and TPA can induced differentiation by different pathways. The amount of basal phospholipase A2 activity was also related to previously found differences in the ability of the clones to develop desensitization to beta-adrenergic hormones or prostaglandin E1. (Author abstract) (25 Refs)

OTHER RELATED STUDIES

86. CHANGES IN AMINO ACID AND SUGAR TRANSPORT ACTIVITY ASSOCIATED WITH MALIGNANT TRANSFORMATION.

Nishino H, Inui K
Dept. Biochemistry, Kyoto Prefectural Medical Univ., Kyoto, Japan
Tanpakushitsu Kakusan Koso; 26(7):881-895 1981

Changes in the membrane transport of amino acids and sugars by transforming cells in many systems were reviewed. Increase in membrane transport is usually due to the increase of transport carriers as the cell membrane changes. To study such mechanism, the method of using membrane vesicles made from purified membrane has been developed and widely applied to the amino acid transport system. This membrane vesicle system eliminates the complication of metabolism associated with the whole cells. Such system is also increasingly applied to to the study of sugar transport as well. Other useful techniques are also developed, such as purification of transport carriers and membrane, and creation of an in vitro system by artificially combining the two components. Some salient results were presented and discussed. (75 Refs) (In JPN)

87. INHIBITORS AND PATHWAYS OF HEPATOCYTIC PROTEIN DEGRADATION.

Seglen PO, Gordon PB, Grinde B, Solheim A, Kovacs AL, Poli A
Dept. Tissue Culture, Norsk Hydro's Inst. for Cancer Res., Norwegian Radium Hosp., Montebello, Oslo 3, Norway
Acta Biol Med Ger; 40(10/11):1587-1598 1981

Activation of hepatocytic protein degradation (PD) by nutritional step-down (amino acid depletion), distinction between lysosomal and non-lysosomal degradation by the use of inhibitors, two non-lysosomal modes of degradation, lysosomal modes of degradation, and purine derivatives: specific inhibitors of autophagic/lysosomal degradation are discussed. Rat hepatocytes isolated by collagenase perfusion, can be maintained as monolayer cultures in a defined, amino acid-rich medium supplemented with dexamethasone and insulin at least 10 days. Protein synthesis and PD are in balance, at a turnover rate of approx 1%. Nine different classes of inhibitors effective on hepatocytic PD are known. In all those cases where comparison has been made the degradation of stable protein is more affected by inhibitors than is the degradation of labile proteins. Data support the contention that different pathways are involved in the degradation of these two classes of protein, the stable protein-preferring pathway being more sensitive to inhibitors. Differential sensitivity towards chymostatin supports the idea that energy-dependent and energy-independent degradation represents two distinct modes of non-lysosomal degradation. Amino acids and other inhibitors of the lysosomal pathway (leupeptin, vinblastine, and propylamine) all inhibit endogenous PD to approx the same final extent, disregarding the lag phase. However, when the degradation of an exogenous endocytosed protein (asialofetuin) is measured, amino acids contrast with the other inhibitors in having no effect. (32 Refs)

88. LEUCINE METABOLISM IN TUMOR AND SELECTED TISSUES OF C57/BL MICE BEARING LEWIS LUNG CARCINOMA (MEETING ABSTRACT)

Chan W, McElhanon S, Kaplan AM, Banks WL
Medical College of Virginia, Richmond, VA, 23298
Fed Proc; 41(3):281 1982

We have previously reported the presence of branched-chain amino acid transferase as well as active LEU oxidation by LL tumor. Considering that LL can grow to 10-20% of the host wt, its metabolism affects that in the host. The purpose of this study is to examine the in vitro LEU utilization by tumor, liver and diaphragm. Tumor inoculated mice and age-matched controls (C) were fed lab chow ad lib for 18-21 days. Tissue slices were incubated in KRB (pH7.4) containing 0.1mM LEU and 14C-1-LEU (SA:25 Ci/mole). After 90 min of incubation, CO2 in the media was liberated, trapped and counted. Cold 50% TCA was added to tissue homogenates to separate the acid soluble and acid insoluble fractions, which were further processed and counted. The results showed active utilization of LEU by the tumor, whose presence increased LEU oxidation (p less than .01) by the diaphragm. The mechanism of the effect warrants further investigation. (no Refs)

89. AUTOCATABOLISM OF SURFACE MACROMOLECULES SHED BY HUMAN MELANOMA CELLS.

Bystryn JC, Perlstein J
Dept. Dermatology, New York Univ. Sch. Medicine, 560 First Ave., New York, NY, 10016
Cancer Res; 42(6):2232-2237 1982

The fate of cell surface macromolecules released by human melanoma cells in vitro was studied. Labeled surface macromolecules released by lactoperoxidase-radioiodinated melanoma cells were incubated with unlabeled cells. It was found that some of these macromolecules were autocatabolized to acid-soluble fragments by the cells which had released them. Degradation did not occur in the absence of cells, was almost completely inhibited at 4 C, and was partially suppressed by cytochalasin B (10 ug/ml) and by some inhibitors of energy production, ie, iodoacetamide (10(-4) M) and a combination of 2-deoxyglucose (18 mg/ml) and 2,4-dinitrophenol (10(-4) M). Radioiodinated surface macromolecules were degraded much more rapidly than radioiodinated serum proteins. Thus, degradation required the presence of cells, was in part an active process, and was selective. These results suggest that one of the pathways for the turnover of surface macromolecules on tumor cells is shedding followed by autocatabolism of the shed material by the cells which they have released. (Author abstract) (33 Refs)

90. HEAT SHOCK PROTEINS AND BIOLOGICAL RESPONSE TO HYPERTHERMIA.

Subjeck JR, Sciandra JJ, Chao CF, Johnson RJ
Dept. Radiation Medicine, Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY, 14263
Br J Cancer; 45(Suppl5):127-131 1982

Thermotolerance is of significant interest because the resistance of pre-heated cells greatly surpasses the resistance of control cells. The induction of protein synthesis following a temperature shock (12 min, 45 C) which produces thermotolerance is examined using SDS polyacrylamide gel electrophoresis. It was observed that the synthetic rates of certain proteins following heat shock significantly exceeded their synthetic rates in control (non-heat shocked) cells. The most strongly induced bands were observed at 68,000, 89,000 and 110,000 daltons. The induction of these proteins was blocked by inhibitors of transcription (actinomycin D) or translation (cycloheximide). This phenomenon strongly resembles the heat shock induction of gene transcription well studied in Drosophila melanogaster. The strong correlation between the kinetics of the induction of these heat shock proteins and the kinetics of thermotolerance induction is discussed. (Author abstract) (17 Refs)

91. INDUCED THERMAL TOLERANCE AND HEAT SHOCK PROTEIN SYNTHESIS IN CHINESE HAMSTER OVARY CELLS.

Li GC, Petersen NS, Mitchell HK
Dept. Radiology, Stanford Univ. Sch. Medicine, Standford Univ., Stanford, CA, 94305
Br J Cancer; 45(Suppl5):132-136 1982

Kinetics of induction of thermal tolerance in Chinese hamster HA-1 cells and effects of heat treatments on recovery of protein synthesis are reviewed with emphasis on the ability of heat shock to induce certain proteins. Acquisition of thermal tolerance in HA-1 cells correlated in time with synthesis of heat shock proteins (hsp) but only poorly with synthesis of a non-hsp proteins. The three proteins whose synthesis was enhanced by heat shock had mol wt of 59 kilodaltons (K), 70 K, and 87 K. The rates of synthesis of the hsp and actin were plotted as a function of incubation time at 37 C after 45 C heat exposure. By 8 hr, the rates of synthesis of 59 K, 79 K, and 87 K proteins and actin were 140%, 160%, 115%, and 53%, respectively. Thus hsp were made under conditions where cells were developing thermal tolerance. The role of hsp in the development of thermal tolerance is discussed. (17 Refs)

92. HUMAN HEAT SHOCK GENE EXPRESSION AND THE MODULATION OF PLASMA MEMBRANE NA+,K+-ATPASE ACTIVITY.

Burdon H, Cutmore CM
Dept. Biochemistry, Univ. Glasgow, Glasgow G12 8QQ, Scotland
FEBS Lett; 140(1):45-48 1982

Evidence is presented to suggest heat shock protein (HSP) gene expression involvement in the modulation of Na+,K+-ATPase activity in HeLa cells exposed to temperature elevation. It is suggested that phosphorylation of Na+,K+-ATPase could lead to its inactivation and its reversal might require the direct association of an HSP or the participation of an HSP in a dephosphorylation reaction. These findings bear upon the use of hyperthermia in human cancer treatment. (26 Refs)

93. PATTERNS FOR NORMAL COLON MUCOSA AND COLON ADENOCARCINOMA COMPARED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS.

Tracy RP, Wold LE, Currie RM, Young DS
Section Clinical Chemistry, Mayo Clinic, Rochester, MN, 55905
Clin Chem; 28(4,part2):915-919 1982

A two-dimensional gel 'map' of the proteins in normal cells and transformed cells may well help establish the presence of putative tumor-specific protein antigens, which can be isolated and used as antigens. We so examined normal colon mucosa and colon adenocarcinoma, using a sensitive silver stain. Samples were promptly prepared after excision by rapidly trimming the tissue to a small, grossly homogeneous piece, which was frozen on solid CO2. Frozen histological sections and permanent mounts were also prepared for several areas, to document the cellular constitution and to grade the carcinoma. Sample preparation for electrophoresis is described. The DNA was pelleted by centrifugation (108,000 X g X 1.5 hr). Little or no pellet was observed, indicating complete solubilization. Many of the tumors contained proteases, liberated during sample preparation, so protease inhibitors were included. A highly reproducible pattern was observed for normal mucosa. Comparison of the gels from paired samples with histological data allows many of the spots to be tentatively assigned to the different cell types present in the mucosa (epithelium, connective tissue, or muscularis mucosa) and, in most tumors analyzed, detection of a series of spots that are not detectable in the pattern for normal tissue. These apparently tumor-associated proteins may be of epithelial cell or stromal origin. (Author abstract) (13 Refs)

94. LYMPHOCYTE, MONOCYTE, AND GRANULOCYTE PROTEINS COMPARED BY USE OF TWO-DIMENSIONAL ELECTROPHORESIS.

Gemmell MA, Anderson NL
Div. Biological Medical Res., Argonne Natl. Lab., Argonne, IL, 60439
Clin Chem; 28(4,part2):1062-1066 1982

We compared cellular proteins from normal human blood lymphocytes, monocytes, and granulocytes, using high-resolution two-dimensional electrophoresis. The leukocytes were isolated from peripheral blood by centrifugation on density step gradients (yielding a fraction of purified granulocytes and a lymphocyte/monocyte mixture) and monocytes were subsequently separated from lymphocytes by virtue of their adherence to plastic. Wright-stained smears indicated that each of the three resulting fractions was 90 to 95% pure. The cells were labeled with [35S]-methionine after various intervals in culture, then solubilized and analyzed by two-dimensional electrophoresis. Although most proteins in each cell type are common to all three, there are nevertheless several specific marker proteins that distinguish one cell type from another. We also examined the appearance of these markers in three lines of cultured cells from humans (GM607, a B-lymphoblastoid line; HL-60, a promyelocytic leukemic line; and 1494, a normal skin fibroblast). (Author abstract) (17 Refs)

95. TWO-DIMENSIONAL ANALYSIS OF HUMAN LYMPHOCYTE PROTEINS: III. PRELIMINARY REPORT ON A MARKER FOR THE EARLY DETECTION AND DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS.

Willard KE
Div. Biological Medical Res., Argonne Natl. Lab., Argonne, IL, 60439
Clin Chem; 28(4,part2):1031-1035 1982

Two-dimensional gel electrophoretic patterns of human peripheral blood leukocytes from 12 patients with infectious mononucleosis were prepared by use of the ISO-DALT system. Before the two-dimensional separation, the leukocytes were purified by Ficoll-Paque gradient centrifugation and labeled overnight with [35S]methionine. Quantitative increases in two proteins were detected in the patterns of infected leukocytes from the patients as compared with controls. Fluorescence-activated cell sorting of leukocytes from normal human peripheral blood before subsequent two-dimensional gel analysis revealed that the dramatic increase in one of these proteins (Inmono:2) could be due to shifts in the population ratios of lymphocytes, monocytes, and granulocytes. In contrast, the appearance in the infected leukocytes of a second protein, Inmono:1 could not be accounted for by cell-population shifts. Increased amounts of these two proteins have been found in every patient studied who had clinically detectable infectious mononucleosis. In addition, a patient who displayed symptoms of infectious mononucleosis but who did not have a positive result in the MONOSPOT test until 3 weeks after our analysis also demonstrated increased relative amounts of these proteins in his leukocyte pattern. (Author abstract) (12 Refs)

96. TWO-DIMENSIONAL GEL ELECTROPHORESIS OF CELL PROTEINS OF CHILDHOOD LEUKEMIA, WITH SILVER STAINING: A PRELIMINARY REPORT.

Hanash SM, Tubergen DG, Heyn RM, Neel JV, Sandy L, Stevens GS, Rosenblum BB, Krzesicki RF

Dept. Pediatrics, Univ. Michigan, Box 66, F6515, Ann Arbor, MI, 48109

Clin Chem; 28(4,part2):1026-1030 1982

We have investigated the potential of two-dimensional electrophoresis for identifying differences in protein patterns between acute lymphocytic (ALL) and acute myelogenous leukemia (AML). We studied blood cells directly isolated from seven children with non-T, non-B ALL and five with AML. Solubilized proteins were electrophoresed with the ISO-DALT system and made visible by silver staining. More than 800 spots were routinely detected in individual gels. Patterns for multiple runs of the same sample were distinguishable. Most of the prominent spots were present in gels from all 12 of the individuals studied. However, substantial variability in spot intensity was observed, even among patients in the same group. Five spots were consistently present in gels of all the AML patients, and either completely absent or substantially decreased in ALL gels. Three other spots were present in ALL but not AML gels. We conclude that this may be a useful tool for characterizing leukemic cells. (Author abstract) (16 Refs)

97. A TWO-DIMENSIONAL GEL ANALYSIS OF AUTOLOGOUS T AND B LYMPHOBLASTOID CELL LINES.

Lester EP, Lemkin P, Lipkin L

Dept. Medicine, Univ. Chicago Sch. Medicine, 950 East 59th St., Chicago, IL, 60637

Clin Chem; 28(4,part2):828-839 1982

We sought polypeptide markers of pathways of lymphoid differentiation by comparing the protein-synthesis patterns of a pair of autologous human lymphoblastoid cell lines: the B-cell line CCRF-SB and the T-cell line CCRF-HSB-2. Cells were cultured in standard medium for 24 or 120 hr, then biosynthetically radiolabeled with [3H]leucine. Whole cell lysates were subjected to two-dimensional gel electrophoresis and patterns of protein synthesis were made visible by fluorography. Replicate cultures showed highly reproducible patterns, but differing culture durations produced alterations in the relative synthetic rates of some proteins. Protein synthetic patterns were analyzed visually and with the GELLAB system for computer analysis of two-dimensional gel images. Qualitative and quantitative differences were found between the T- and B-cell patterns. GELLAB detected and accurately paired most of the well-defined spots in each image and constructed a unified data base from data on our initial gels. Statistical analysis of this data revealed several marker proteins, the relative synthetic rates serving to distinguish the T-cell line from the B-cell line. Such computerized analysis of protein synthetic patterns of malignant cells appears likely ultimately to provide important diagnostic information regarding human malignancies. (Author abstract) (22 Refs)

98. HUMAN PROTEINS SENSITIVE TO NEOPLASTIC TRANSFORMATION IN CULTURED EPITHELIAL AND FIBROBLAST CELLS.

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Clin Chem; 28(4,part2):949-954 1982

We resolved the [35S]methionine polypeptides synthesized by normal [lung fibroblasts (WI-38), amnion cells] and transformed [SV40 transformed WI-38, AMA (spontaneously transformed amnion cells)] cultured human cells, using two-dimensional gel electrophoresis under conditions in which about 1,300 polypeptides could be reproducibly separated. These studies demonstrated important changes in the relative proportions of several polypeptides that are present both in normal and transformed cells. Of a total of 400 common polypeptides that we quantitated for each cell type, 53 (22 basic and 31 acidic) varied by 40% or more in both cell pairs. Among these, we have identified vimentin (IEF 26), cyclin (IEF 49), and a tropomyosin-related polypeptide (IEF 52). No new major polypeptide was detected in the transformed cells, at least at the level of resolution currently achieved by this technique. Similar but qualitative studies of [32P]orthophosphate-labeled proteins revealed that, of 250 analyzed, only 7 common phosphoproteins, including phosphovimentin (IEF 26e), varied consistently in both cell pairs. These results strengthen our previous conclusion that transformation results in changes in the relative proportions of polypeptides synthesized in normal and transformed cells rather than in the appearance of new polypeptides in transformed cells. (Author abstract) (28 Refs)

99. UP-DATED CATALOGUE OF HELA CELL PROTEINS: PERCENTAGES AND CHARACTERISTICS OF THE MAJOR CELL POLYPEPTIDES LABELED WITH A MIXTURE OF 16 14C-LABELED AMINO ACIDS.

Bravo R, Celis JE

European Molecular Biology Lab., Postfach 10.2209, Meyerhofstrasse 1, 6900 Heidelberg, W. Germany

Clin Chem; 28(4,part2):766-781 1982

A total of 1,357 polypeptides [946 acidic (isoelectric focusing) and 411 basic (nonequilibrium pH-gradient electrophoresis)] from human HeLa cells have been separated and catalogued with use of high-resolution two-dimensional gel electrophoresis. Of these polypeptides, 1,266 were detected by labeling cells with [35S]methionine, while the rest were revealed by silver staining or by labeling with a mixture of 16 14C-labeled amino acids. For convenience, all these polypeptides have been numbered and are indicated in a large fold-out protein map. The percentages of some of the major 14C-labeled proteins have been determined, and for some we list a few characteristics such as: variation during the cell cycle; cellular distribution in cytoplasts and karyoplasts; presence in Triton- and salt-extracted cytoskeletons; and phosphorylation and sensitivity to neoplastic transformation. (Author abstract) (14 Refs)

100. SELECTIVE INHIBITION OF A CYCLIC NUCLEOTIDE INDEPENDENT PROTEIN KINASE (G TYPE CASEIN KINASE) BY QUERCETIN AND RELATED POLYPHENOLS.

Cochet C, Feige JJ, Pirollet F, Keramidas M, Chambaz EM
Biochimie Endocrinienne, INSERM U-244, CNRS ERA 942, Universite Scientifique et Medicale, CERMO, BP 53 X, 38041 Grenoble, France

Biochem Pharmacol; 31(7):1357-1361 1982

The effect of quercetin and a number of structurally related phenolic compounds upon the activity of three different purified protein kinases was examined. Whereas the catalytic subunit of a cyclic AMP-dependent protein kinase and an A type (using only ATP) cyclic nucleotide-independent casein kinase (CKA) were not affected, a G type (using GTP as well as ATP) casein kinase (CKG) was selectively inhibited by several bioflavonoid structures. Kinetic studies showed that quercetin behaved as a competitive inhibitor toward the nucleotidic substrate and exhibited a high affinity for the ATP (Ki = 0.75 uM) and GTP (Ki = 0.22 uM) site of the enzyme. Considering the CKG inhibitory potency of a series of flavonoid, cinnamic acid and coumarin derivatives, it is suggested that the biological activity lays upon a common structural feature involving a phenolic ring bearing a side chain with conjugated double bonds and an oxygenated function, as found in the coumaroyl residue. These observations suggest that quercetin and related compounds may lead to a shift in intracellular protein phosphorylations by selectively inhibiting a particular type of protein kinase activity (CKG). It remains to be established whether this process may contribute to the mechanism of action of flavonoids upon cellular metabolism, particularly in the case of malignant cells. (Author abstract) (15 Refs)

101. COVALENT PROTEIN BINDING OF REACTIVE ADRIAMYCIN METABOLITES IN RAT LIVER AND RAT HEART MICROSOMES.

Scheulen ME, Kappus H, Nienhaus A, Schmidt CG
Dept. Internal Medicine (Cancer Res.), West German Tumor Center, Univ. Essen Medical Sch., Hufelandstrasse 55, D-4300 Essen 1, W. Germany

J Cancer Res Clin Oncol; 103(1):39-48 1982

Covalent binding of 3H-labeled adriamycin metabolites to bovine serum albumin and microsomal protein is demonstrated in an aerobic incubation system with rat liver and rat heart microsomes, respectively, using exhaustive organic solvent extraction and gel chromatography. Covalent protein binding was dependent on active microsomes, NADPH, and oxygen and was inhibited by reduced glutathione and other sulfhydryl compounds. The anthracycline moiety was spectrophotometrically evidenced in the adriamycin metabolite(s) covalently bound to protein. Thus, enzymatic activation of adriamycin in the heart with consecutive covalent protein binding of reactive adriamycin semiquinone radicals may contribute to adriamycin cardiotoxicity. (Author abstract) (48 Refs)

102. WHAT ARE ISOZYMES TELLING US ABOUT GENE REGULATION IN CANCER?

Weinhouse S

Fels Res. Inst., Temple Univ. Sch. Medicine, Health Sciences Center, Philadelphia, PA, 19140

JNCI; 68(3):343-349 1982

Roles of isozymes in cancer, examples of isozyme alteration in hepatomas, fetal isozyme expression in hepatomas, tumor-associated proteins, ectopic isozymes, loss of adult liver enzymes, and significance of isozyme alterations are reviewed. Striking changes in properties of serum aldolase in several human diseases have been observed. The serum enzyme resembled that of muscle aldolase in some muscular diseases and resembled that of liver aldolase in hepatitis or liver glycogen storage disease. In liver tumor patients the serum had the muscle isozyme. As with aldolase B, glucokinase is lost to varying degrees in hepatomas, depending on the growth rate and degree of dedifferentiation, and is replaced completely in fast-growing, poorly differentiated hepatomas by one or more of the other three hexokinase isozymes that are present in fetal liver. At least three distinct genotypic forms of alkaline phosphatase are present in human tumors and in plasma of cancer patients. An example of the resurgence of a fetal isozyme in tumors is gamma-glutamyl-transpeptidase. This enzyme is bound to the plasma membrane and is normally low in adult rat liver but very high in fetal liver and chemically induced hepatomas. Studies in which pyruvate kinase (PK) was specifically inhibited in Ehrlich ascites cells provide further suggestive evidence that the replacement of a tightly controlled hepatic PK by a high activity of the fetal form may be responsible for the high glycolytic activity of rapidly growing, poorly differentiated hepatomas. (57 Refs)

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AUTHOR INDEX WITH CITATION NUMBERS

Amano T 30
Anderson NG 32
Anderson NL 94
Appella E 59
Arnold EA 39
Astrup EG 80
Avila J 10
Bachoski G 45
Ballard FJ 72
Banes AJ 21
Banks WL 88
Barnstable CJ 11
Barsukov LI 47
Basciano LK 43
Baumann H 57
Becerra SP 2
Bergelson LD 47
Bernd A 18
Bernier D 66
Bigotti A 56
Blalock JE 51
Bodmer JG 52
Bodmer WF 52
Bondy PK 84
Boutwell RK 80
Boynton AL 36
Brandes D 39
Brandes MJ 39
Bravo R 98
Bravo R 99
Brinkley SB 20
Broadhead G 31
Brynskov J 48
Burdon H 92
Busch H 3
Busch H 6
Busch RK 3
Busch RK 6
Bystryn JC 89
Canellakis ZN 84
Carlson P 60
Carraway NG 19
Celis JE 98
Celis JE 99
Chambaz EM 100
Chan PK 6
Chan W 88
Chao CF 90
Chapman JF 33
Chen KY 81
Cheresh DA 34
Chretien P 66
Cittanova N 31
Cochet C 100
Cohen EP 63
Cohen LH 1
Colaco C 10
Coleman MS 7
Coll J 10
Comings DE 19
Conrad DH 60
Conrad NC 78
Cooper HL 71
Cowing D 68
Crumpton MJ 52
Cuocolo R 79
Currie RM 93
Cutmore CM 92
Dabbous MK 20
Dahlenburg GW 72
Davis DF 77
De Kretser TA 52
De Luca LM 40
De Martino G 79
Debuire B 28
Dermer GB 33
Dickerman HW 26
Distasio JA 34
Dohi Y 30
Dubois GC 59
Dulis BH 73
Dyatlovitskaya 47
Edstrom S 67
Edwards JJ 32
Ekman L 67
El-Torky M 20
Eldredge D 57
Engler R 31
Evans DL 46
Fan K 65
Fantuzzi LF 5
Feige JJ 100
Ferrone S 56
Finkelstein R 68
Foidart JM 24
Frakes RL 6
Francis GI 72
Fridovich SE 44
Fujio H 30
Gazitt Y 84
Gemmell MA 94
Gendler SJ 62
Gesell MS 39
Ghosh L 78
Giddings S 64
Gimsing P 48
Gleiber WE 25
Gmeiner B 37
Goldstein IJ 22
Goodman DS 41
Gordon PB 87
Gozes I 11
Grey HM 73
Grigorova AM 31
Grinde B 87
Halliday JW 29
Hanash SM 53
Hanash SM 96
Haney L 20
Harrap KR 76
Henshaw EC 61
Herrara JM 38
Herve F 31
Hewitt AT 24
Heyn RM 96
Hirayama A 30
Honeysett JM 83
Hopkins M 42
Hughes TK 51
Ichikawa Y 13
Imai K 56
Insel PA 83
Inui K 86
Irimura T 49
Isaacs JT 39
Isersky C 43
Johnson RJ 90
Juhasz P 75
Kakita K 64
Kamath SK 78
Kaplan AM 88
Kappus H 101
Karlberg I 67
Keramidas M 100
Kimata K 24
Kleeberg U 75
Kleinman HK 24
Klinger W 75
Kloppel TM 73
Kobayashi T 30
Kohrs MB 78
Kondo K 30
Kosaki G 30
Kovacs AL 87
Krakower G 45
Krzesicki RF 96
Kubo RT 73
Kurano R 54
Kute TE 42
Landry J 66
Ledford BE 77
Lee V 14
Lemkin P 97
Lennox RW 1
Lester EP 97
Li GC 91
Lin LC 28
Liotta LA 23
Lipkin L 97
Liu AY 81
Lodish HF 44
Lopez DM 34
Lovett EJ 22
Lundholm K 67
MacManus JP 36
Maidhof A 18
Marceau N 66
Martens C 63
Martin GR 23
Martin GR 24
Mastro AM 55
Mastropaolo W 61
McCormick AM 38
McElhanon S 88
McGhee K 76
McTigue JJ 15
Mechanic GL 21
Mehta RG 42

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In this issue:

- NUCLEOPROTEINS 1-3
- CYTOPLASMIC AND CONTRACTILE PROTEINS 4-17
- PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS 18-32
- BINDING/ RECEPTOR PROTEINS 33-41
- MEMBRANE/ SURFACE PROTEINS 42-56
- STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS 57-61
- EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM 62-66
- DIETARY AND NUTRITIONAL EFFECTS 67-70
- POLYAMINES - SYNTHESIS AND METABOLISM 71-73
- OTHER RELATED STUDIES 74-79

NUCLEOPROTEINS

1. POLY(ADP-RIBOSYL)ATION OF CHROMOSOMAL PROTEINS IN THE HELA S3 CELL CYCLE.

Tanuma S, Kanai Y
Dept. Physiological Chemistry, Teikyo Univ., Sagamiko, Kanagawa 199-01, Japan
J Biol Chem; 257(11):6565-6570 1982

Change in the level of poly(ADP-ribosyl)ation during the cell cycle of HeLa S3 cells was examined in three different in vitro systems: isolated nuclei, chromatin, and poly(ADP-ribose) polymerase extracted from isolated nuclei, and by the direct immunofluorescent antibody technique in situ. In isolated nuclei, after release from thymidine-hydroxyurea double block, the level of poly(ADP-ribosyl)ation was low in the S phase and increased in the late G2 phase with a peak in the M phase. In chromatin, the level began to increase in the mid-late S phase, was maintained at a high level through the G2 phase, decreased in the M phase, and became basal during the G1 phase. The activity of poly(ADP-ribose) polymerase extracted from isolated nuclei was relatively high throughout the cell cycle and was highest in the G2 phase. Similar changes during the HeLa S3 cell cycle were observed in the three systems

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after release from Colcemid block. In synchronously growing HeLa S3 cells in situ, nuclei in the G2-M phase were stained strongest for poly(ADP-ribose) by the immunofluorescent staining. These results were consistent with the peak of poly(ADP-ribosyl)ation in the G2-M phase observed in the nuclear system in vitro. (Author abstract) (32 Refs)

2. BENZO(A)PYRENE- BINDING PROTEINS OF HAMSTER EMBRYO CELL NUCLEI: COMPARISON OF NUCLEAR ISOLATION PROCEDURES.

MacLeod MC, Mansfield BK, Selkirk JK
Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
J Cell Biochem; 18(4):507-513 1982

Two techniques of purifying nuclei from which benzo(a)pyrene (BP)-binding proteins are isolated are compared. One bound nuclear protein, BP32, is preferentially lost from nuclei purified by a modification of the Chauveau method. BP32 is either intranuclear or tightly associated with the nucleus. Major loss of BP32 occurs not as a result of hydrodynamic shearing produced by centrifugation through 2.2 M sucrose, but as a result of osmotic shock when the 2.2 M sucrose pellet is resuspended in a solution of much lower osmolarity (0.25 M sucrose). Due to the similarity of BP32 to nucleoplasmin from frog oocytes in terms of nuclear leakage, the possibility that BP32 may be the mammalian counterpart of nucleoplasmin is currently being explored. The functional significance of polycyclic aromatic hydrocarbon binding to nuclear proteins remains unknown. (20 Refs)

3. NORMAL AND NEOPLASTIC HUMAN CELLS HAVE DIFFERENT HISTONE H1 COMPOSITIONS.

Tan KB, Borun TW, Charpentier R, Cristofalo VJ, Croce CM
Wistar Inst. Anatomy and Biology, Philadelphia, PA, 19104
J Biol Chem; 257(10):5337-5338 1982

The H1 histone is the least conserved of the five major groups of histone proteins. There are as many as five subtypes of H1 histone. These H1 subtypes occur in different amounts in different animal species and also show tissue specificity. Normal and neoplastic tissues from the same animal, eg rat or calf, contain the same H1 subtypes but in different relative amounts. Because H1 subtypes exhibit tissue specificity, it is therefore difficult to identify the changes in their composition that are associated with neoplasia reported in studies utilizing tissues of different origins. Tissue culture cells and their in vitro transformed neoplastic counterparts, on the other hand, offer an excellent system in which to study these changes because the cells are derived from the same origin. We have examined normal and neoplastic human cells and found a relationship between the H1 composition and the ability of the cells to form tumors in nude mice. The ratio of H1A to H1B in normal human cells is considerably lower than that in neoplastic cells and this ratio increases with the increased ability of the cells to form tumors in nude mice. (Author abstract) (14 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

4. PHOSPHORYLATION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN LEUKEMIC CELLS.

Elias L, Longmire J, Wood A, Ratliff R
Dept. Medicine, Univ. New Mexico Sch. Medicine, Albuquerque, NM, 87131
Biochem Biophys Res Commun; 106(2):458-465 1982

Because of the specific involvement of protein phosphorylation in malignant transformation, the possibility that terminal deoxynucleotidyl transferase (TdT) might act as a physiological substrate for leukemic cellular protein kinase (PK) was investigated. NALM-6 (TdT-positive human lymphoblastic leukemia) cells were labeled with 32-PO4 and TdT was recovered from the lysate by phosphocellulose chromatography followed by immunoprecipitation. Electrophoresis revealed a major labeled band of mol wt 59,000 corresponding to the high mol wt form of TdT characteristic of human leukemic cells. The phosphoprotein was recovered and subjected to phosphoroaminoacid analysis, which disclosed 85% phosphoserine and 15% phosphothreonine. Purified calf thymus TdT was phosphorylated in vitro by crude and partially purified human leukemic cell PK. TdT phosphorylation by leukemic cell extracts and semi-purified PK was not stimulated by cAMP. Phosphorylation of TdT was shown to increase activity significantly, particularly in the presence of high concentrations of riboATP, as occur intracellularly. These results suggest a mechanistic link between two enzymatic systems, each of which is of largely unknown function but both of which are prominently associated with leukemia. TdT phosphorylation may be part of a coordinated general pathway pertaining to control of gene transcription and protein synthesis which is appropriately activated in proliferating normal and malignantly transformed cells. (20 Refs)

5. ORNITHINE DECARBOXYLASE FROM HEPATOMA CELLS AND A VARIANT CELL LINE IN WHICH THE ENZYME IS MORE STABLE.

Pritchard ML, Pegg AE, Jefferson LS
Dept. Physiology, Coll. Medicine, Milton S. Hershey Medical Center, Pennsylvania State Univ., Hershey, PA, 17033
J Biol Chem; 257(10):5892-5899 1982

The half-life of ornithine decarboxylase in HMOA cells, a variant cell line derived from hepatoma tissue culture (HTC) cells, was 5 to 10 hr compared to 14 min in the parental cell line. The half-lives of two other rapidly turning over proteins, S-adenosylmethionine decarboxylase and tyrosine aminotransferase, as well as the turnover of total cellular protein, were the same in the two types of cells, suggesting that other proteins did not share the alteration in turnover observed with ornithine decarboxylase. The possible existence of a mutant enzyme was investigated by purifying ornithine decarboxylase 8,000-fold from HTC and HMOA cells, the key step being a pyridoxamine-phosphate affinity column. The enzymes from both cell types behaved in an identical manner throughout the purification procedure, were equally thermolabile, and were inhibited in a similar manner by antiserum to ornithine decarboxylase and by ornithine decarboxylase antizyme. The Km of the two purified enzymes for L-ornithine were approx the same (0.04 mM) as were the Kis for putrescine (0.14 mM). Studies employing [14C]difluoromethylornithine, a radioactive irreversible inhibitor that specifically labeled ornithine decarboxylase, showed that the enzyme from both cell types had a subunit Mr of 54,000. The amounts of ornithine decarboxylase in the soluble fractions of logarithmically growing HTC and HMOA cells, as determined by binding of the inhibitor, were 3.5 and 11.0 ng/mg protein, respectively. It is concluded that the increased half-life of ornithine decarboxylase in HMOA cells is not due to a more stable form of the enzyme, the alternative possibility being that a specific deactivation system for the enzyme is altered in these cells. (Author abstract) (48 Refs)

6. SIALYLTRANSFERASE ACTIVITY IN LEUKEMIA.

Sasaki R, Takaku F, Lin YL, Bollum FJ
Dept. Hematology, Jichi Medical Sch., 311-1 Yakushiji Minamikawachi-Machi, Kawaschi-Gun, Tochigi 329-04, Japan
Leuk Res; 6(2):197-202 1982

Assayed sialyltransferase activity (STA) in blasts from various types of leukemia patients was compared with the level of activity in mature lymphocytes or granulocytes. STA in blasts from acute lymphoblastic leukemia (ALL) patients was markedly lower (1.68 +-1.23 picomole; pmol/mg protein) than those (6.18 +-2.22 pmol/mg protein) of lymphocytes from the peripheral blood of normal volunteers. Also, in blasts from terminal transferase (TdT)-positive chronic myeloid leukemia (CML) patients in blasts crisis, a low level of the enzyme activity compatible to those of ALL patients was observed. STA was significantly increased in blasts (1.21 +-0.38 pmol/mg protein) from patients with acute non-lymphoblastic leukemia including acute myeloblastic leukemia, acute myelomonocytic leukemia, and acute promyelocytic leukemia, compared to the level of mature granulocytes (0.53 +-0.32 pmol/mg protein). However, only a slight difference was observed between the level of STA in the granulocytes from CML patients in chronic phases and in the granulocytes from normal volunteers. In TdT-negative CML in blast crisis, STA (2.11 +-0.88 pmol/mg protein) was significantly higher than those of mature granulocytes from normal controls, whereas no significant difference of the enzyme activity was noted between the blasts from TdT-positive CML in blast crisis and from ALL. (27 Refs)

7. DIRECT CYTOCHEMICAL LOCALIZATION OF CATALYTIC SUBUNITS DISSOCIATED FROM CAMP-DEPENDENT PROTEIN KINASE IN REUBER H-35 HEPATOMA CELLS. I. DEVELOPMENT AND VALIDATION OF FLUORESCINATED INHIBITOR.

Fletcher WH, Byus CV
Div. Biomedical Sciences, Dept. Biochemistry, Univ. California, Riverside, CA, 92521
J Cell Biol; 93(3):719-726 1982

A specific and sensitive procedure has been developed that reliably localizes intracellular sites of free catalytic unit (C) dissociated from cAMP-dependent protein kinase. The method is based on a FITC conjugate (F:PKI) of affinity column-purified heat-stable protein inhibitor (PKI) of free C. The fidelity of this cytochemical probe was determined using cultures of Reuber H-35 hepatoma cells that had been stimulated for 2 hr with 0.1 mM DBcAMP, or with diluent, then fixed with anhydrous acetone at-30 C. In these preparations the F:PKI probe complexed with free C in cytoplasm, nucleolus, and, to a minor extent, in nucleoplasm. Binding of the F:PKI molecule to free C was competitively diminished by arginine analogues, guanidinium HCl and polyarginine, each used over a 2-log dose range. When the inhibitor's arginine residues were blocked by reaction with cyclohexanedione it no longer inhibited phosphotransferase activity of free C, and when fluorescinated it failed to localize C in stimulated cells. Similarly, when F:PKI was preabsorbed with excess pure C it no longer functioned as a cytochemical stain. Affinity column-purified antibody to free C also reduced significantly the ability of F:PKI to complex with C in cell cultures stimulated with 0.1 mM DBcAMP. One microgram of antibody reduced by approx 10% the binding of F:PKI to all cell compartments while 5 ug of antibody diminished binding by greater than 50%. Together, these results indicate that the F:PKI binds specifically, perhaps exclusively, to the catalytic units of cAMP-dependent protein kinase. The cytochemical procedure, unlike its biochemical counterparts, is able to locate the dissociation of cAMP-dependent protein kinase in individual cells of functionally or histologically complex cultures. Also, it reveals variations in the time- and dose-dependent activation of the kinase amongst clonal cells stimulated with cyclic nucleotide analogues or hormones. (Author abstract) (34 Refs)

8. DIRECT CYTOCHEMICAL LOCALIZATION OF CATALYTIC SUBUNITS DISSOCIATED FROM CAMP-DEPENDENT PROTEIN KINASE IN REUBER H-35 HEPATOMA CELLS. II. TEMPORAL AND SPATIAL KINETICS.

Byus CV, Fletcher WH
Div. Biomedical Sciences, Dept. Biochemistry, Univ. California, Riverside, CA, 92521
J Cell Biol; 93(3):727-734 1982

The activation of cyclic AMP-dependent protein kinase results in the release of the catalytic subunit which as the free enzyme possesses phosphotransferase activity for a variety of specific protein substrates. Using a cytofluorometric technique we monitored the appearance of free catalytic subunit in Reuber H35 hepatoma cells in culture after incubation with N(6)-1'-O-dibutyryl-cyclic AMP (DBcAMP), 8-bromoadenosine-3':5'-cyclic monophosphate (8-BrcAMP), and glucagon. The cytochemical method employs the heat-stable inhibitor of the free catalytic subunit which has been conjugated to fluorescein isothiocyanate (F:PKI). Here we studied the temporal and spatial kinetics of the free catalytic subunit following activation of cAMP-dependent protein kinase by increasing concentrations of DBcAMP,8-BrcAMP, and glucagon. Under similar conditions protein kinase activation was also assessed biochemically in H35 cell supernatants by assaying the protein kinase activity ratio. Incubation of the hepatoma cells with DBcAMP led to an increase in the activity ratio. During the same period using the F:PKI probe, a significant increase in cytoplasmic and nucleolar fluorescence indicative of the release of the free catalytic subunit was coincidentally observed. Discernible nucleoplasmic fluorescence did not occur until 1 hr after the addition of DBcAMP. Cultures incubated with 8-BrcAMP exhibited a more rapid activation of the protein kinase measured cytochemically. Cultures incubated with 8-BrcAMP had significantly increased cytoplasmic and nucleolar fluorescence compared to unstimulated cells. The results reported herein suggest that both cyclic nucleotide analogues and a polypeptide hormone lead to the activation of cAMP-dependent protein kinase in similar intracellular compartments in Reuber H35 hepatoma cells. The appearance of protein kinase catalytic subunit in the nucleolus also serves to support earlier reports which suggest a biological role for this enzyme in the nucleus. (33 Refs)

9. ALTERATION OF THE REGULATORY SUBUNIT OF TYPE 1 CAMP-DEPENDENT PROTEIN KINASE IN MUTANT Y1 ADRENAL CELLS RESISTANT TO 8-BROMOADENOSINE 3':5'-MONOPHOSPHATE.

Doherty PJ, Tsao J, Schimmer BP, Mumby MC, Beavo JA
(c/o Schimmer) Banting and Best Dept. Medical Res., Univ. Toronto, Toronto, Ontario M5G 1L6, Canada
J Biol Chem; 257(10):5877-5883 1982

The biochemical basis for cellular resistance to ACTH and cAMP was examined in a family of mutant clones (Kin) derived from the Y1 adrenocortical tumor cell line. Extracts from the Kin mutants bound [3H]cAMP poorly when compared with extracts from parental Y1 cells. The degree of impairment in cAMP-binding activity among Kin mutants corresponded with the degree of impairment in cAMP-dependent protein kinase activity demonstrated previously. Impaired cAMP-binding activity in several Kin mutants also was observed with the photoreactive probe, 8-azido-[32P]cAMP, and appeared to result from alterations in the Type 1 cAMP-dependent protein kinase. The holoenzymes from the parent Y1 and two mutant Kin clones were separated into catalytic and Type 1 regulatory (RI) subunits. Homologous and heterologous holoenzymes were formed from the isolated subunits and assayed for cAMP-dependent protein kinase activity. Analysis of the reconstituted holoenzymes indicated that the RI subunit was responsible for the altered cAMP-dependent protein kinase activity observed in the two Kin mutants. Y1 and mutant Kin clones were labeled with L-[35S]methionine and the RI subunits were immunoprecipitated. When analyzed on isoelectric focusing gels, the immunoprecipitates from the two mutants contained an acidic variant form of RI. This variant species of RI appears to be related to the altered phenotype of the Kin mutants, and may be responsible for the observed impairments in cAMP-dependent protein kinase activity and cAMP-binding activity. (Author abstract) (34 Refs)

10. PRESENCE OF CALMODULIN IN PARATHYROID ADENOMAS.

Oldham SB, Lipson LG, Tietjen GE
Dept. Medicine, Univ. Southern California Sch. Medicine, 2025 Zonal Ave., Los Angeles, CA, 90033
Miner Electrolyte Metab; 7(5):273-280 1982

A significant percent of parathyroid adenomas demonstrate decreased sensitivity to the suppressive effects of calcium on parathyroid hormone secretion. The recognition that calmodulin mediates a large number of calcium-dependent cellular events suggests that calmodulin may play a key role in the normal regulation by calcium of parathyroid activity. We therefore undertook to determine if parathyroid adenomas contained calmodulin and if so, if the calmodulin content of adenomas could be correlated with the serum concentrations of calcium and immunoreactive parathyroid hormone (IPTH) in hyperparathyroid patients prior to surgery. Calmodulin was assayed by activation of a calmodulin-stimulatable phosphodiesterase (PDE) obtained from rat brain. We found PDE-stimulating activity in each of six adenoma extracts examined. The stimulation had the following properties, which are characteristic of calmodulin-mediated processes: (1) it required the presence of calcium; (2) the dose response to adenoma extract and human red blood cell calmodulin were parallel, and (3) the stimulation was phenothiazine-inhibitable . The amount of calmodulin present in the adenoma extracts ranged from 0.4 to 1.25 units/ug protein. No correlation was found between the calmodulin content of the adenomas and the presurgical levels of either serum calcium or serum IPTH. (Author abstract) (31 Refs)

11. A BIOCHEMICAL STUDY ON THE SOFT TISSUE TUMORS: ESPECIALLY ON NEUROFILAMENT PROTEINS.

Sakihara H, Hanaoka H, Maruya M, Iri H, Mori M, Horiuchi Y
Dept. Orthopedics, Keio Univ., Tokyo, Japan
Nippon Seikeigeka Gakkai Zasshi; 55(9):1087 1981

By polyacrylamide gel electrophoresis, two neurofilament-like bands (mol wt 200,000 and 160,000) two tubulin bands (mol wt 56,000 and 53,000), and an actin-like band (mol wt 43,000) were detected in samples of human nevus ischiadicus, neurinoma, neurofibroma, and malignant neurinoma. A thin 200,000 band and a dense 43,000 actin band were seen in leiomyoma sample, whereas a dense 200,000 and a thin 160,000 band were observed in desmoid. In lipoma, 16,000, 53,000, and 43,000 bands were missing. In two dimensional electrophoresis, most spots were common to peripheral nerve and neuro-cancers. Histologically and electron microscopically, neurite-like matters were detected in neurinoma and neurofibroma. (no Refs) (In JPN)

12. PARTIAL PURIFICATION OF MEMBRANE-BOUND GLUTATHIONE PEROXIDASE FROM CARDIAC MITOCHONDRIA (MEETING ABSTRACT)

Katki AG, Myers CE, Hendrickson MF
Tufts-New England Medical Center, Boston, MA, 02111
Med Pediatr Oncol; 10(1):102 1982

As part of our investigation of the role of lipid peroxidation in doxorubicin toxicity, we are examining the detoxification of H2O2 in cardiac mitochondria. We reported the presence of membrane-bound glutathione peroxidase which is independent of dietary selenium. We now report partial purification and some properties of this new enzyme. It was found that digitonin fractionation of mitochondria in 220 mM mannitol-70 mM sucrose-2 mM Hepes, pH 7.5, with mitochondrial protein to digitonin ratio of 3 gave the optimum recovery of the membrane-bound enzyme. DE-52 cellulose ion exchange chromatography with 0 to 0.1 m NaCl gradient in the above-mentioned buffer with 0.25 mM GSH (growth stimulating hormone) gave 3 fractions: Fraction B, and active enzyme fraction, followed by Fraction G, which was an inhibitor of the enzyme activity, followed by active Fraction D. The ratio of optical density at 260 nm to 280 nm suggest between 12-16% nucleic acid in all fractions; also, the active fraction and the inhibitor fraction show hyperchromic shift, suggesting the presence of double-stranded structure of the nucleic acid component. On cooling, the activity is also decreased, suggesting confirmational changes in the nucleic acid moeity of the molecule. Both the active and the inhibitor fractions were stable to heat (100 degrees) for as much as 2 to 3 hours with concomitant increase in enzyme activity. Heating the pooled fraction followed by DE-52 ion exchange chromatography did not resolve the active fraction from inhibitor. However, a substantial amount of 260 nm absorbing material was eluted after the active fraction. In conclusion, the membrane-bound glutathione peroxidase isolated from cardiac mitochondria seems to be a nucleoprotein. (1 Ref)

13. CHANGES IN MYOSIN DURING DIFFERENTIATION OF MYELOID LEUKEMIA CELLS.

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Dept. Cytochemistry, Chest Disease Res. Inst., Kyoto Univ., Sakyo-ku, Kyoto, Kyoto 606, Japan
J Biochem (Tokyo); 91(4):1363-1372 1982

Changes in cellular myosin were followed during the differentiation into macrophages of a myeloid leukemia cell line (M1) which can be induced by conditioned medium (CM) from a rat embryo culture. To extract the myosin, we used three different procedures, all of which gave a lower yield of myosin for the differentiated than for the undifferentiated M1 cells. This low extractability we attributed to increased binding of the myosin to the plasma membrane. Taking the different extractabilities into consideration, we calculated the myosin contents in the total cellular protein from the densitometry of SDS-polyacrylamide electrophoresis, 0.6% for the untreated M1 cells and 1.0% for the differentiated ones. The three ATPase activities of the M1 cell myosin were in the order, K+-EDTA-=Ca++- much greater than Mg++-ATPase in the presence of 0.6 M KCl, whether or not there was treatment with CM. Myosin was purified through fractionation with 25-55% saturated ammonium sulfate, then gel filtration with Sepharose 4B followed by affinity chromatography on F actin-Sepharose 4B. The M1 cell myosin consists of one heavy chain (H) and three light chains (L1, L2, L3), with molecular ratios of L1 + L2/H = 1 and L3/H = 1. The ratio of L1/L2 was about 1.2 for the untreated M1 cells, but it decreased to about 0.7 after differentiation. (Author abstract) (30 Refs)

14. PURIFICATION OF THE INTERMEDIATE FILAMENT PROTEIN VIMENTIN FROM EHRLICH ASCITES TUMOR CELLS.

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J Biol Chem; 257(10):5536-5543 1982

Two procedures have been used for the purification of the intermediate filament protein vimentin from Ehrlich ascites tumor (EAT) cells grown in vitro. In one procedure, vimentin was first incorporated into residual cell structures by extracting EAT cells with Triton X-100 in the presence of Mg++ and then solubilized with low ionic strength buffer in the absence of Mg++. In the second procedures, which led to the purification of both vimentin and the Ca++-activated proteinase specific for this intermediate filament protein, both components were immediately solubilized by extracting EAT cells with hypotonic buffer in the absence of Triton X-100. In both procedures, a vimentin-enriched fraction was obtained by precipitating vimentin with (NH4)2SO4 at 23% saturation. Following lyophilization and delipidation with chloroform/methanol, vimentin was purified by column chromatography on DEAE-Sepharose Cl 6B in 6 M urea. The first procedure resulted in a 33-fold purification of vimentin in a yield of 39%. The same method was also used to prepare [3H]vimentin from EAT cells labeled with 3H-amino acids and resulted in a specific activity of 8 x 10(5) cpm/mg purified vimentin. By two-dimensional polyacrylamide gel electrophoresis only one protein species was seen following staining with silver The purified protein had a subunit mol wt of 58,000 and consisted of several isoelectric variants (pI approx 5.3). In the absence of urea and at low salt concentration the purified vimentin was highly aggregated and had a sedimentation coefficient of 8 s. (Author abstract) (48 Refs)

15. IN VITRO STUDY OF TUBULIN POLYMERIZATION: EFFECTS OF ATP, VINBLASTINE AND ISAXONINE.

Coelho R, Istin M
Departement de Biologie, CEN Saclay, Laboratorie d'Etudes du Metabolisme des Medicaments, F 91191 Gif-sur-Yvette Cedex, France
Nouv Presse Med; 11(16):1227-1232 1982

The dual action of vinblastine on tubulin, namely disruption and aggregation, is analyzed by turbidimetry. The existence of two different binding sites for the alkaloid is confirmed. The high affinity site binds vinblastine at low concentrations, at which no effect is observed on depolymerized tubulin, but where pre-formed microtubules are disrupted. The low affinity site is associated with tubulin aggregation. The binding constants of these two sites have been evaluated. Isaxonine (N-isopropyl-amino-2 pyrimidine) partially inhibits the effects of vinblastine, both on microtubule disruption and tubulin aggregation. (Author abstract) (35 Refs) (In FRE)

16. IDENTIFICATION OF CYTOKERATIN ANTIGENS IN NOVIKOFF ASCITES HEPATOMA.

Schmidt WN, Pardue RL, Tutt MC, Briggs RC, Brinkley BR, Hnilica LS
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Proc Natl Acad Sci USA; 79(10,part1):3138-3142 1982

Further characterization of three polypeptide antigens present in Novikoff ascites hepatoma (mol wt 39,000, 49,000, and 56,000-p39, p49, and p56, respectively), has established the identity of these proteins as cytokeratins. Two-dimensional gel electrophoresis revealed that each of the three proteins exists in several isoelectric forms, all characteristic of the keratins. The antigens p39 and p56 had amino acid compositions compatible with this family of proteins, and immunofluorescence microscopy localized the antigens on elaborate filament arrays indicative of the cytokeratins. Antibodies monospecific for the p39 antigen, purified by using affinity chromatography, were used to demonstrate that protein p39 was specific for Novikoff hepatoma cells. These results suggest that the cytokeratins contain cell-specific species that can be useful as markers for differentiation and neoplasia. (Author abstract) (50 Refs)

17. THE RELATIONSHIP OF GLIAL FIBRILLARY ACIDIC PROTEIN TO THE SHAPE, MOTILITY, AND DIFFERENTIATION OF HUMAN ASTROCYTOMA CELLS.

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Exp Cell Res; 139(1):145-157 1982

Glial fibrillary acidic protein (GFAP), a protein largely limited to astrocytes, was studied in relation to the shape, motility, and differentiation and malignancy of astrocytoma cells in tissue culture by use of time-lapse photography and the immunoperoxidase method. A relationship was observed between the shape of astrocytes and the distribution of GFAP. A relationship was also observed between the motility of astrocytes and GFAP. We hypothesize that GFAP filaments may be inhibitory to great plasticity of motion but not to extension-retraction movements. During mitosis GFAP was sparse at the spindle and in intercellular bridges. Colcemid caused GFAP to disappear from processes and peripheral parts of the cell and to become concentrated near the nucleus. In cultures derived from malignant tumors, undifferentiated and large multinucleated cells usually showed sparsity of GFAP, but occasional well differentiated stellate or spindle-shaped cells containing abundant GFAP were seen. Conversely, although cultures derived from benign tumors may have scattered less well differentiated cells, the differentiated cells with well developed processes were most densely stained and account for the high concentration of GFAP in tissue from these tumors. (54 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

18. IMMUNOGLOBULIN G BIOSYNTHESIS IN A HUMAN LYMPHOBLASTOID CELL LINE. DIFFERENCES BETWEEN MEMBRANE-BOUND AND SECRETORY FORMS OF GAMMA CHAINS.

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Eur J Biochem; 124(1):79-87 1982

The cultured human B lymphoblastoid cell line Maja synthesizes two forms of the gamma heavy chain of immunoglobulin G (IgG) that differ in apparent mol wt. The lower-mol-wt form is secreted into the culture medium as a water-soluble product in association with light chains and comigrates on dodecyl sulfate polyacrylamide gels with serum IgG gamma chains. The higher-molecular-wt form is not detected in culture supernatants. In distinction to the secreted form, the higher-mol-wt form is labeled by a lipophilic, photoactivatable nitrene and is inserted asymmetrically in a transmembrane orientation into rough microsomes. It is concluded that Maja cells synthesize secretory (gammas) and membrane-associated (gammam) forms of IgG heavy chains. Both forms of the gamma heavy chain are glycosylated, and can contain one or two asparagine-linked glycan units. The gammam and gammas heavy chains differ by about 10,000 in apparent mol wt. This difference resides exclusively in the polypeptide moiety. Although part of the difference comprises a transmembrane peptide and a cytoplasmic tail of apparent mol wt about 2,000 for gammam chains, a substantial segment of unique peptide is most probably present on the non-cytoplasmic side of the bilayer. The ionophore monensin inhibits the intracellular transport of gammas and gammam chains at a stage when they are sensitive to the enzyme endo-beta-N-acetylglucosaminidase H. In contrast, HLA-A and HLA-B antigens reach a stage at which they are insensitive to this enzyme in the presence of monensin, although their surface expression is inhibited by the ionophore. The implications of these results for the intracellular transport of membrane-associated glycoproteins are discussed. (Author abstract) (38 Refs)

19. CORRELATION OF COLLAGENASE SECRETION WITH METASTATIC COLONISATION POTENTIAL IN NATURALLY-OCCURRING MURINE MAMMARY TUMOURS (MEETING ABSTRACT)

Tarin D, Hoyt BJ, Evans DJ, Ydenberg RC
Department of Histopathology, University of Oxford, Oxford, England
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

In this communication we report evidence demonstrating the secretion of a true mammalian collagenase active against Type I collagen by naturally-occurring mammary tumors of the mouse and show that tumors capable of heavily colonizing the lungs secrete significantly greater quantities of this enzyme than those with low pulmonary colonization potential or than non-neoplastic proliferating (eg lactating) mammary tissue. Plasminogen activator was secreted in greater quantity by tumors than by normal tissues but there was no significant difference in the amount produced by tumors with high or low pulmonary colonization potentials. (no Refs)

20. REACTIVITY WITH PATIENT ANTIBODIES OF PARTIALLY PURIFIED GP40 ANTIGEN FROM IMMUNE COMPLEXES IN BURKITT'S LYMPHOMA AND NASOPHARYNGEAL CARCINOMA.

Gazitt Y, Klein G, Sulitzeanu D
Lautenberg Center for General and Tumor Immunology, Hebrew Univ. Hadassah Medical Sch., Jerusalem, Israel
Int J Cancer; 29(6):645-651 1982

A glycoprotein antigen (gp40) was previously identified as a major component of immune complexes isolated from sera of patients with Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). In the present work gp40 was partially purified from a pool of BL/NPC sera. Sera of patients with BL and NPC as well as sera of patients with other malignant and non-malignant diseases were tested for antibodies against gp40, using a double antibody radioimmunoassay. Practically all normal sera and sera of patients with non-malignant diseases had antibodies capable of binding radioiodinated gp40. In contrast, sera of patients with BL or NPC had very low binding, while sera of patients with other malignant diseases had intermediate binding values. The low binding activity of BL/NPC sera was shown to be due to inhibition by excess gp40 present in such sera. Effusions of patients with breast or ovarian cancer also contained demonstrable amounts of gp40. The origin of gp40 is still unknown. (Author abstract) (25 Refs)

21. ACUTE PHASE PROTEINS-DYNAMICS IN INFLAMMATORY OR TUMOR-BEARING ANIMALS.

Okubo H, Shibata K, Ishibashi H, Kudo J, Ikuta T, Tsuda-Kawamura K
First Dept. Medicine, Faculty Medicine, Kyushu Univ., Fukuoka 812, Japan
Fukuoka Igaku Zasshi; 73(3):146-153 1982

Alpha1-acid glycoprotein (a1AGP), alpha1-antitrypsin (a1AT), and alpha2-macroglobulin (a2MG) were studied and compared with respect to time-course changes in serum concentration following induced inflammation and time-course patterns in tissue distribution of the proteins. Results indicated that a1AGP and a1AT, which accumulate in pathological tissues, may modulate the inflammatory process or tumor proliferation, while a2MG may function mainly in the circulation in the early phase of inflammation. (21 Refs)

22. FIBRONECTIN IN EXPERIMENTAL GRANULATION TISSUE.

Holund B, Clemmensen I, Junker P, Lyon H
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Acta Pathol Microbiol Scand [A]; 90(3):159-165 1982

The temporal appearance of fibronectin in experimental granulation tissue has been studied using the immunoperoxidase technique on material fixed in formaldehyde, embedded in paraffin and pretreated with pepsin. Furthermore, the relationship between the distribution of fibronectin and connective tissue fibers, demonstrated as either argyrophilic or red by the van Gieson method, has been investigated. Fibronectin was demonstrated as part of the fibrinous network in the sponge before the formation of granulation tissue. In the development of granulation tissue fibronectin was seen in the initial cellular stage codistributed with newly formed fibers, first the argyrophilic fibers and later with those stained red by the van Gieson method, too. With maturation of the granulation tissue, predominated by these fibers, the amount of fibronectin was gradually reduced. The presence of fibronectin in the sponge already before the formation of granulation tissue suggests that fibronectin in the initial stage of inflammation derives from plasma. The increase in amount of fibronectin corresponding to the cellular (fibroblastic) proliferation and the formation of fibers, both argyrophilic and van Gieson positive, indicates that fibronectin in young granulation tissue is synthesized by fibroblastic cells. The relationship between the morphologically demonstrated argyrophilic fibers and van Gieson positive fibers and the biochemical Type III and Type I collagens is discussed. (Author abstract) (39 Refs)

23. HYALURONECTIN IN NORMAL HUMAN SKIN AND BASAL CELL CARCINOMA.

Delpech A, Delpech B, Girard N, Boullie MC, Lauret P
Laboratoire d'Histologie, Hopital Charles Nicolle, 1, Rue de Germont, 76031 Rouen Cedex, France
Br J Dermatol; 106(5):561-568 1982

The localization of hyaluronectin has been studied in normal skin and in basal cell carcinoma. In fetal skin it is abundant in the dermis but absent from the epidermis, and in adult skin it is totally absent except in the hair sheaths and bulbs. In basal cell carcinoma it is abundant only in the stroma reaction. The presence of this protein in mesenchymatous tissues seems to be linked to zones of physiological or neoplastic proliferation. (Author abstract) (18 Refs)

24. THE ROLE OF A SERUM DNA-BINDING PROTEIN (64DP) IN THE DIAGNOSIS OF MALIGNANT NEOPLASMS.

Mitomi T, Nakasaki H, Tajima T, Ogoshi K, Tsuda M, Katsunuma T
Dept. Surgery, Sch. Medicine, Tokai Univ., 259-11 Isehara, Japan
Int Adv Surg Oncol; 5:245-259 1982

Role of the DNA-binding protein with mol wt 64,000 dalton (64DP) as a tumor marker in diagnosing and monitoring patients with malignant disease is described. 64DP was measured with column sodium dodecyl sulfate-polyacrylamide gel electrophoresis and a single radial immunodiffusion method. The elevation of serum 64DP in 96 patients with malignant disease over that observed in normal controls or in patients with nonneoplastic disease appeared quite significant. Serum 64DP levels in 26 patients with stomach carcinoma were categorized according to clinical stages. No clear correlation was observed between the levels of 64DP and the clinical stage. Twenty-nine patients were subjected to serial determination of serum 64DP to monitor the response to treatment. Serum 64DP showed a drop approx 3 wk after resectional treatment. The 64DP level did not appear to decrease to normal value even after complete removal of the malignant lesion by radical gastrectomy. Eleven patients with recurrent stomach carcinoma were tested for 64DP. The level ranged from 31-220 ug/ml in these patients. Approx half of the patients did not show elevation of 64DP levels. Patients with carcinoma of other organs showed preoperatively high values of 64DP with subsequent decreases 3-4 wk after successful surgical treatment. Analysis of the amino acid composition of 64DP indicated that it is rich in leucine, glutamic acid, and aspartic acid. Amino acids in lower amounts were tryptophan and cysteine plus cystine. (14 Refs)

25. PARTIAL PURIFICATION OF BONE-INDUCING SUBSTANCE FROM A MURINE OSTEOSARCOMA (BFO).

Takaoka K, Yoshikawa H, Shimizu N, Ono K, Nakada Y, Amiya K
Dept. Orthopedics, Osaka Univ., Osaka, Japan
Nippon Seikeigeka Gakkai Zasshi; 55(9):1097-1098 1981

An osteogenic factor, isolated and purified twice on Sephacryl S-200 gel chromatography from BFO osteosarcoma, was subjected to SDS polyacrylamide gel electrophoresis. Two bands with a mol wt 22,500 and 19,000 were detected. Sugar content measured with phenol-sulfuric acid method was about 4%. The osteogenic factor appears to be a glycoprotein with a mol wt of about 20,000. (no Refs) (In JPN)

26. THE TRANSFORMATION-SENSITIVE GLYCOPROTEIN LAMININ IS AN AGGLUTININ (MEETING ABSTRACT)

Kennedy DW
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Med Pediatr Oncol; 10(1):100 1982

Laminin is a high molecular weight glycoprotein involved in the adhesion of a variety of epithelial cells to type IV collagen of basement membranes. The protein consists of at least two polypeptide chains of 220,000 and 440,000 daltons joined to each other by disulfide bonds. Laminin is found to agglutinate formalinized sheep erythrocytes at protein concentrations of under 1 ug/ml. This finding provides a simple model system for analyzing laminin-mediated adhesion and for searching for its cell surface receptor in competitive inhibition experiments. Laminin-mediated hemagglutination is markedly inhibited by highly negatively charged phospholipids and by purified solubilized gangliosides. The inhibition is dose-dependent and competitive; hemagglutination by 4 ug/ml laminin is half-maximally inhibited by less than 1 uM phosphatidic acid and by less than 5 uM gangliosides. The order of inhibition by gangliosides is GD1a greater than GT1b greater than GM1. A series of other lipids, ganglioside constituents, monosaccharides, glycosaminoglycans, and glycoproteins are either less effective or without detectable competitive inhibitory activity. These results suggest that hypothesis that laminin mediates normal epithelial cell adhesion by binding to a cell-surface receptor that may contain a negatively charged cell-surface component such as a ganglioside or another highly charged lipid. (no Refs)

27. IMMUNOFLUORESCENT ANALYSIS OF THE SPECIFIC TYPES OF COLLAGEN FOUND IN ASSOCIATION WITH PULMONARY NEOPLASMS (MEETING ABSTRACT)

Madri J, Carter D
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71st Annual Meeting of the International Academy of Pathology (United States-Canadian Division), March 1-5, 1982, Boston, Massachusetts. International Academy of Pathology 95 pp., 1982.

The fibrosis associated with pulmonary carcinomas has evoked considerable interest over the years, and controversy surrounds the relationship between the two. It has been suggested that the fibrosis is: (1) a tumor product, (2) a host reaction to the growth of the tumor, (3) elicited or actively provoked by the tumor, or (4) a preexisting scar that is involved in the development of the tumor. To study the relationship of the fibrosis to the tumors, the five specific types of collagen and laminin were analyzed in three pulmonary tumors and compared with the fibrosis which occurs in interstitial pulmonary fibrosis that is not associated with a neoplasm. An atypical pseudolymphoma of the lung was found to have no appreciable fibrosis associated with the tumor. Two adenocarcinomas were studied. One was a poorly differentiated adenocarcinoma with extensive associated fibrosis, and the other showed extensive interstital fibrosis with a bronchioloalveolar pattern. In both adenocarcinomas, the fibrosis was composed of types III and V collagen, whereas the residual pulmonary parenchyma exhibited the usual staining patterns having a punctate localization of types I and III collagen and basement membrane localizations of types IV and V collagen and laminin. The relative increase and localization of type V collagen is similar to that observed in interstital fibrosis which was not associated with tumor. None of the staining suggested that the fibrosis was a tumor product. (no Refs)

28. LOSS OF BASEMENT MEMBRANE COMPONENTS BY INVASIVE TUMORS BUT NOT BY THEIR BENIGN COUNTERPARTS (MEETING ABSTRACT)

Barsky SH, Siegal GP, Jannotta F, Liotta LA
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71st Annual Meeting of the International Academy of Pathology (United States-Canadian Division), March 1-5, 1982, Boston, Massachusetts. International Academy of Pathology 95 pp., 1982.

Highly purified antibodies to three ubiquitous components of the basement membrane zone (type IV collagen, type V collagen, and laminin) were applied to both fresh frozen and fixed, paraffin-embedded permanent sections in a variety of invasive lesions, carcinomas in situ, and their 'look-alike' benign counterparts. These included such lesions as invasive breast carcinoma (scirrhous), in situ ductal carcinoma, and sclerosing adenosis; pancreatic carcinoma and pancreatitis; squamous cell carcinoma, Bowen's disease, and pseudoepitheliomatous hyperplasia; endometrial carcinoma and adenomyosis; prostatic carcinoma and benign prostatic hyperplasia; colonic adenocarcinoma and villous adenoma without invasion; and other lesions of the invasive, in situ, and noninvasive benign category. By both immuonfluorescence and immunoperoxidase techniques, all benign and in situ lesions showed intact basement membrane with linear staining of type IV collage, type V collagen, and laminin. In contrast, these three basement membrane components were absent in invasive carcinomas. In cases of in situ carcinoma with microinvasion, there was thinning, fragmentation, and disruption of the basement membrane in the foci of microinvasion. In some invasive tumors, especially well-differentiated carcinomas and in some sarcomas like synovial sarcoma, tiny discrete foci of extracellular basement membrane staining could be demonstrated with one or more of these antibodies, suggesting abortive attempts at reformation of basement membrane after initial invasion. (no Refs)

29. CHEMOTAXIS OF GRANULAR LEUKOCYTE: ITS BIOCHEMICAL AND MORPHOLOGICAL MECHANISM.

Hirashima M, Hirostu Y, Hayashi H
First Dept. Pathology, Kumamoto Univ. Sch. Medicine, Kumamoto, Japan
Nippon Rinsho; 39(8):2701-2708 1981

Two neutrophil chemotactic factors (NCF) are known: the primary factor leucoegresin (mol wt 140,000) and the secondary factor (mol wt 14,000) a derivative of C5. Both are thermoresistant. Artificial peptides, such as f-Met-len-phe, also serve as NCF. Others were also found, but were thought to be less important. Eosinophilic chemotactic factor (ECF) consists mainly of thermolabile early factor (mol wt 500) and thermolabile delayed factor (mol wt 70,000). The early factor may be the ECF-A (ECF of anaphylaxis) or a similar molecule. The delayed factor can be further separated into two different antigens: a and b. Various other factors were also found. All basophilic chemotactic factors studied so far are the results of the in vitro experiments, and no studies have yet been performed with a natural mediator. The two types of leukocyte movement are the random movement occurring in a medium without concentration gradient of the chemotactic factors (CF), and the directional chemotactic movement that initiates the gradient of CF concentration. The latter movement is initiated when CF binds CF receptors on the membrane to form lamellipodium as the forward section. The binding of CF to the CF receptor triggers several biochemical reactions, acting on microfilaments and microtubules, which are moved around within the cell due to gradient changes of Ca++ and Na+. (45 Refs) (In JPN)

30. EVIDENCE OF A PHOSPHORYLATED PROTEIN SPECIFIC FOR HUMAN COLON CARCINOMA CELLS.

Munsch N, Raux H, Fondaneche MC, Burtin P
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Biochem Biophys Res Commun; 106(1):87-93 1982

Two human tumor cell lines, HT29 and Caco2, and the mammary tumor cell line MDA -MB-231 synthesize in culture a 42 kilodalton (42K) protein detectable by immunoprecipitation. This protein, which is phosphorylated in the two colon tumor cell lines, is not detectable by antisera against normal adult colon, suggesting that the 42K protein represents a tumor specific antigen. Addition of a differentiating agent, retinoic acid, reduces the growth of the tumor cells by 50%, but has no effect on the expression of the 42K protein. (10 Refs)

31. SYNTHESIS AND SECRETION OF ALPHA-FETOPROTEIN AND ACUTE-PHASE ALPHA2-MACROGLOBULIN BY ZAJDELA RAT ASCITES HEPATOMA.

Morinaga T, Andrews GK, Panrucker DE, Lorscheider FL, Tamaoki T
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Oncodev Biol Med; 3(1):23-29 1982

Zajdela rat ascites hepatoma cells have been reported to be 'nonsecretors' of alpha-fetoprotein (AFP). Because of the potential of such cells in the study of defective secretory mechanisms of AFP and other serum proteins, we conducted a detailed investigation of the production of AFP in Zajdela cells growing in vivo. Three approaches were used: radioimmunoassay of intracellular and extracellular AFP and assays of AFP mRNA by molecular hybridization and cell-free translation. Radioimmunoassay showed that the AFP concentration in the hepatoma cells was extremely low (1.2 ug/g) as compared to known AFP-producing tissues such as fetal liver (600 ug/g). The assays of AFP mRNA by hybridization and translation supported the low level of AFP production in Zajdela cells, and we consider such cells to be poor producers of AFP and therefore not suitable for the study of defective mechanisms for AFP secretion. The Zajdela tumor cell intracellular concentration of acute-phase alpha2-macroglobulin (APalpha2M) measured by radioimmunoassay was also found to be low (3.0 ug/g) compared to liver of Zajdela-bearing rats (80 ug/g). In view of these low levels of synthesis of AFP and APalpha2M in Zajdela hepatoma cells, little biological significance may be attached to the apparent nonsecretion of these onco-fetal proteins. (Author abstract) (13 Refs)

32. CLINICAL APPLICATIONS OF PROTEIN DETERMINATIONS IN BIOLOGICAL FLUIDS OTHER THAN BLOOD.

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Clin Chem; 28(5):1093-1102 1982

Over the past decade, significant improvements in immunochemical and electrophoretic techniques have enabled collection of heretofore unavailable data on proteins in biological fluids, greatly increasing our understanding of protein physiology in the various body compartments and providing the foundation for clinical use of protein analysis in body fluids. The most striking advance has been in the diagnosis of demyelinating disease through the use of serum/cerebrospinal fluid protein ratios and the morphological evaluation of immunoglobulin banding patterns. These laboratory tests are now considered obligatory for any patient in whom demyelinating disease is suspected as the cause of neurological dysfunction. Cerebrospinal fluid protein data can also be helpful in quantitating the permeability of the blood/cerebrospinal fluid barrier in many inflammatory or infectious central nervous system disorders. Assays of individual proteins in urine can help distinguish between different types of proteinuria, and can give quantitative data on the selectivity of the glomerulus and the reabsorbing capacity of the tubules. The protein content of saliva, synovial fluid, and milk has also been well characterized, and is clinically applicable to a wide range of disorders. (Author abstract) (153 Refs)

BINDING/ RECEPTOR PROTEINS

33. PROGESTIN RECEPTORS IN PROLACTIN AND GROWTH HORMONE PRODUCING TUMOURS IN RATS.

Naess O, Haug E, Attramadal A, Gautvik KM
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Acta Endocrinol (Copenh); 100(1):25-30 1982

Progesterone and corticosterone have a similar effect on the production of growth hormone (GH) and prolactin (Prl) by pituitary tumor cells (GH3 cells) in culture. Previously we have shown that progesterone had a high affinity for the glucocorticoid receptors in these cells. Progesterone may therefore exert its effects through binding to the glucocorticoid receptor. The aim of the present study was to investigate if the GH3 tumor cells and an estrogen induced pituitary tumor, which also produce GH and Prl, possess specific receptors for progesterone. Both the GH3 tumors and the estrogen induced pituitary tumor were in fact found to possess cytoplasmatic receptor molecules for progesterone by using the potent progestin R5020 as a marker. Isoelectric focusing revealed one binding component (pH 5.9), which was of protein nature. The binding was of high affinity (KD 2 x 10(-9) mol/l). In the estrogen induced tumor, the maximal binding was 70 fmol/mg cytosol protein. In female rats with GH3 tumors the binding was 55 fmol/mg cytosol protein. Priming of the animals with 1 mg estradiol-valerate increased the binding to 116 fmol/mg cytosol protein, whereas very little binding was found in GH3 tumors from rats castrated 7 days before sacrifice. The receptors in the estrogen induced pituitary tumor and the GH3 tumors exhibited high affinity for R5020 and progesterone, whereas corticosterone had no significant affinity for the receptors. Using exchange assay, it was demonstrated that the cytoplasmic progestin receptors could be translocated to the nucleus after administration of progesterone to the animals. Thus, the presence of specific progesterone receptors, different from the glucocorticoid receptors, strongly indicates that the effects of progesterone on GH and Prl production are mediated through the progesterone receptors. (Author abstract) (21 Refs)

34. CHARACTERIZATION OF INSULIN RECEPTORS IN HUMAN PROMYELOCYTIC LEUKEMIA CELL HL60.

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Biochem Biophys Res Commun; 106(2):574-581 1982

The presence of receptors for 125I-insulin on HL60 human promyelocytic leukemia cells with specificity analogous to that observed on mature hemopoietic cells and other human cell types is reported. Half-maximal binding was observed at 20 min incubation. When HL60 cells were incubated with 2 x 10-10M 125I-insulin together with increasing concentrations of nonlabeled insulin for 2 hr, a competitive displacement of labeled insulin was seen. Mean insulin receptor concentration was estimated to be around 45,000 per cell. Porcine and human insulin which have the same biological activity displaced porcine 125I-insulin with the same efficiency while porcine proinsulin which has only 2-5% biological activity was 100 less effective than insulin is displacing 125I-insulin. Neither porcine C-peptide nor porcine glucagon caused measurable displacement of 125I-insulin. These results indicate the presence of specific binding sites for insulin on immature human myeloid cells. Properties of the HL60 insulin receptor resembled those described for other insulin binding cells. (28 Refs)

35. CELL CYCLE VARIATION IN 125I-LABELED EPIDERMAL GROWTH FACTOR BINDING IN CHEMICALLY TRANSFORMED CELLS.

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Cancer Res; 42(7):2633-2638 1982

The chemically transformed AKR-MCA and C3H/MCA-58 cell lines spontaneously arrest growth in the G1 phase of the cell cycle due to nutrient deficiency when grown to saturation density in medium with 10% fetal bovine serum. An examination of 125I-labeled epidermal growth factor (EGF) binding in rapidly growing and G1-arrested cells showed that the G1-arrested chemically transformed cells also have a 10-to 20-fold reduction in the amount of 125I-labeled EGF binding relative to the same cells in the rapidly growing state. Recovery of receptor activity by addition of serum-free medium was inhibited by actinomycin D and cycloheximide, suggesting that new messenger RNA synthesis as well as increased protein synthesis is necessary for the recovery of EGF binding. A comparison of EGF binding in C3H/MCA-58 cells and the transformed parent line in the rapidly growing state showed the same approx level of receptor activity. The rapidly growing AKR-MCA cells had approx one-tenth the amount of EGF binding as did the rapidly growing nontransformed parent line. Analysis of data showed a 10-fold greater number of receptors in the

AKR-2B cells relative to the AKR-MCA cells with a lesser difference in receptor affinity. The chemically transformed BP-3T3, like the other two chemically transformed lines, was also demonstrated to arrest growth spontaneously due to nutrient deficiency with an associated 100-fold decrease in EGF binding. Rapidly growing BP-3T3 cells had only slightly less 125I-labeled EGF binding than did the nontransformed parent line in the rapidly growing state. The data indicate that one mechanism for reduction of EGF binding in chemically transformed cells is the propensity of these cells to arrest growth in G1 at saturation density due to low-mol-wt nutrient deficiency, a state associated with decreased EGF binding. (25 Refs)

36. CLINICAL SIGNIFICANCE OF NUCLEAR ANDROGEN RECEPTORS (ARN) IN PROSTATE CANCER (MEETING ABSTRACT)

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Med Pediatr Oncol; 10(1):98 1982

The objectives of this study were to evaluate retrospectively (a) the importance of nuclear androgen receptors in patients with prostatic carcinoma in determining the clinical response of these patients to endocrine therapy; (b) the possible correlation of nuclear androgen receptors with tumor differentiation and (c) the comparison of ARn in patients with benign and malignant prostatic disease. Nuclear androgen receptors were quantitated in 40 malignant and 35 benign specimens using protamine sulfate. Sixty-three percent (26/41) of carcinomas were well differentiated (WD) while 24% and 12% were moderately well differentiated (MWD) and poorly differentiated (PD), respectively. ARn (greater than 50 fmoles/g tissue) were detected in 75% of malignant and 77% of benign neoplasms, respectively; there was no correlation between tumor grade and ARn. The mean ARn levels were higher in benign than in malignant neoplasms. ARn presence was independent of ARc (cytosolic androgen receptors); 75% (14/19) of ARc positive (greater than 3 fmoles/mg protein) and 76% (16/21) of ARc negative patients, respectively, had greater than 50 fmoles/g tissue of ARn. Twenty-nine patients have been evaluated for the relationship between ARn and the clinical response to endocrine therapy. Eighteen of 21 patients (86%) with ARn greater than 50 fmoles/g tissue responded favorably to endocrine therapy. In those patients with ARn less than 50 fmoles/g tissue the response was only 25% (2/8). These preliminary results indicate that nuclear androgen receptors may be clinically useful in selecting patients for endocrine therapy. (1 Ref)

37. COMPARISON OF THE BINDING PROPERTIES OF CYTOSOLIC RECEPTORS FOR 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN VARIOUS MAMMALIAN SPECIES (MEETING ABSTRACT)

Gasiewicz TA, Rucci G
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Toxicologist; 2(1):145 1982

The binding of TCDD and its congeners to a cytosolic receptor protein appears to correlate with their ability to elicit toxic effects in mammals. Differences in species susceptibility to TCDD may be related to alterations in the binding properties of these receptors. Utilizing a hydroxylapatite assay system, we determined the number of receptors, apparent equilibrium dissociation constants (KD), and the ability of TCDD congeners to bind to hepatic and thymic cytosolic receptors from male guinea pigs, rats, hamsters, C57BL/6J, and B6D2F1/J mice. Hepatic receptor concentrations ranged from 23 to 74 fmol/mg cytosolic protein. Those species (guinea pig, rat) which have been found to be most sensitive to TCDD-induced thymic atrophy showed the highest levels of thymic receptors as compared to B6D2F1/J mice and hamsters which showed the lowest levels. In the liver there appeared to be a general relation between receptor affinity towards TCDD and the acute LD50 values in these species. Hepatic receptors from the guinea pig showed the highest affinity while those from B6D2F1/J mice and hamsters demonstrated lower affinities. A similar relationship was observed in the thymus. Also, the ability of various TCDD congeners to bind to hepatic receptors generally decreased with increasing acute LD50 values. (no Refs)

38. MENSTRUAL CYCLE DEPENDENT VARIATIONS OF BREAST CYST FLUID PROTEINS AND SEX STEROID RECEPTORS IN THE NORMAL HUMAN BREAST (MEETING ABSTRACT)

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Joint Meeting of the Society of Surgical Oncology and Society of Head and Neck Surgeons, April 18-23, 1982, Marco Island, Florida. The Society of Surgical Oncology and the Society of Head and Neck Surgeons 265 pp., 1982.

Normal breast and endometrium have distinct menstrual cycle dependent variations in morphology. While menstrual cycle variations of estrogen receptor (ER) and progesterone receptor (PR) have been reported for human endometrium, studies of the breast have been hampered by a lack of samples from a 'normal', non-diseased population. Two glycoproteins, gross cystic disease fluid protein (CDP) and non-receptor progesterone binding protein (PBP) were shown to correlate with ER, PR and degree of breast carcinoma differentiation. We analyzed extracts for ER, PR, PBP and CDP in 56 strictly-defined normal breast tissues. Receptors were quantitated by SDGA for ER or DCCA for PR. Radioimmunoassay was used to quantitate, levels of CDP and PBP. Menstrual cycle dependence was observed for ER, PR, CDP and PBP. Mean ER levels were significantly higher during days 3-7 than at other times in the cycle, as was the number of specimens with ER greater than 3 fmole/mg protein. Higher PR levels and PR values greater than 10 were noted more frequently during day 8-14 than other periods. Both CDP and PBP had significantly increased mean values during day 8-14, low levels days 15-20 and intermediate levels for the other intervals. The cyclic variations in CDP and PBP suggest that these proteins may be useful as indicators of intact ER control. These data also indicate that until sex steroid receptor variations during the menstrual cycle are more fully characterized, it would seem prudent to evaluate the ER status of premenopausal patients by sampling their tumors during the proliferative phase of the menstrual cycle. (no Refs)

39. RAPID TEMPERATURE-DEPENDENT TRANSFORMATION OF THE THYROTROPIN-RELEASING HORMONE-RECEPTOR COMPLEX IN RAT PITUITARY TUMOR CELLS.

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J Biol Chem; 257(10):5462-5470 1982

Thyrotropin-releasing hormone (TRH) binds to specific receptors on GH4C1 rat pituitary tumor cells. The rate of dissociation of [3H]TRH from cell monolayers was much faster (t1/2 less than 1 min) when binding had taken place at 0 C than when binding had taken place at 37 C (t1/2 = 18 min). In isolated membranes the t1/2 values for dissociation were 2 and 26 min after binding at 0 and 23 C, respectively. TRH first formed a complex with rapid dissociation kinetics; the TRH - receptor complex was then converted at elevated temperatures to a form with slow dissociation kinetics. This conversion required 1 hr at 23 C in isolated membranes but was complete within 5 min at 23 to 37 C in intact cells. When [3H]TRH initially bound to cells, 80% of the peptide could be removed by a brief rinse with cold 0.2 M acetic acid/0.5 M NaCl. At 37 C the TRH-receptor complex was transformed to a state where 80% was resistant to the acid/salt rinse within 10 min. This transformation was not reversible and could not be demonstrated in isolated membranes. The rate and extent of conversion of the [3H]TRH-receptor complex to an acid/salt-resistant form were independent of TRH concentration but highly dependent on temperature. Conversion of the TRH-receptor complex was not inhibited by metabolic or cytoskeletal poisons, calcium blockers, lysomotrophic agents, or transglutaminase, protein synthesis, or calmodulin inhibitors. These data suggest that TRH initially binds to a form of the receptor from which the peptide dissociates rapidly. The complex is then converted in a temperature-dependent fashion to a form with slow dissociation kinetics; this can occur in cells or membranes. In intact cells at elevated temperatures, the TRH receptor complex undergoes a further transformation to become acid/salt resistant, and these events all occur within 10 min of hormone binding. (Author abstract) (41 Refs)

40. CALCIUM: CALMODULIN AND CANCER.

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Fed Proc; 41(7):2289-2291 1982

When several fast-growing Morris hepatoma tissue lines are compared with normal adult liver tissue, the following observations are made: calmodulin activity is increased in the cytoplasm and decreased in the membranes of the tumor cells. Total calcium is increased three- to fivefold in the tumors. Cyclic AMP phosphodiesterase activity is increased, whereas cyclic GMP phosphodiesterase activity is decreased. In addition, several of the fast-growing Morris hepatoma tissue lines have a new calcium-binding protein that is not observed in adult liver tissue. It is probable that the Ca++-calmodulin complex is very active in these rapidly growing tumors. (Author abstract) (21 Refs)

41. CYTOSOLIC AND NUCLEAR BINDING PROTEINS FOR 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN THE RAT THYMUS.

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Biochim Biophys Acta; 716(1):16-23 1982

The existence of a high-affinity, low-capacity 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-binding species was demonstrated in cytosol from rat thymus. Tests indicated that it was a protein. An excess of unlabeled 2,3,7,8-tetrachlorodibenzofuran or beta-nephthoflavone displaced [3H]TCDD from the binder whereas phenobarbital, pregnenolone-16alpha-carbonitrile or dexamethasone did not compete. The apparent dissociation constant (Kd) of the [3H]TCDD-binder complex was determined to 0.36 nM and the apparent max amount of binding sites (Bmax) to 68 fmol/mg of cytosolic protein. When analyzed by sucrose density-gradient centrifugation at high ionic strength, the [3H]TCDD-binder complex sedimented at 4-5 S; at low ionic strength the complex sedimented more rapidly, probably due to aggregation. All these data support the interpretation that the demonstrated cytosolic TCDD-binder represents the receptor protein for TCDD. Following iv administration of [3H]TCDD, a low-capacity [3H]TCDD-macromolecule complex was extractable from thymic cell nuclei and was also identified as a protein. The nuclear [3H]TCDD-protein complex sedimented at 4-5 S at high ionic strength. Furthermore, a max uptake of [3H]TCDD in thymic nuclei was observed simultaneously with a decline in cytosolic radioactivity (at 3 h post-injection). These findings suggest that the nuclear [3H]TCDD-protein complex represented [3H]TCDD-receptor complex translocated from the cytoplasm. In conclusion, the rat thymus contains a cytosolic TCDD receptor at a concentration similar to that of the rat hepatic receptor. However, in vivo experiments showed that the nuclear uptake of [3H]TCDD (expressed as dpm/mg DNA) in the thymus was only about 6% of that in liver. Further studies are needed for an understanding of the mechanism behind this discrepancy. (28 Refs)

MEMBRANE/ SURFACE PROTEINS

42. ASYMMETRICAL SURFACE IGG ON MOPC-21 PLASMACYTOMA CELLS CONTAINS ONE MEMBRANE HEAVY CHAIN AND ONE SECRETORY HEAVY CHAIN.

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J Immunol; 128(6):2416-2421 1982

Membrane proteins from the B lymphomas WEHI-231 and 2PK3 and from the plasmacytomas MPC-11 and MOPC-21 were radioiodinated in situ by the lactoperoxidase method and were subjected to two-dimensional (nonreduced, reduced) polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Few heavily labeled membrane proteins were composed of disulfide-bonded subunits. One such protein (mw 200,000 intact and 116,000 reduced) shared some properties with the PC-1 alloantigen, although it was not conclusively identified. A second major disulfide-bonded protein (mw 200,000 intact and 95,000 reduced) has been identified previously as the receptor for transferrin. Membrane immunoglobulins of WEHI-231 (IgM) and 2PK3 (IgG2a) had the expected subunit structure, whereas membrane immunoglobulin was not detected on MPC-11. In contrast, surface IgG1 of MOPC-21 appeared to consist almost entirely of hybrid molecules containing one membrane gamma1 chain and one secretory gamma1 chain. This hybrid IgG molecule appeared to exist in both monomeric and dimeric forms. It is concluded that (i) the synthetic and assembly mechanisms of secretory and membrane IgG1 are shared; (ii) there are no special mechanisms to prevent pairing of membrane and secretory gamma1 chains; (iii) the presence of one hydrophobic tail is sufficient for membrane insertion of gamma1 chains; and (iv) the C-terminal extension cysteine residues of membrane gamma1 chains in hybrid IgG molecules are either unpaired or may allow the formation of hybrid IgG dimers. (Author abstract) (37 Refs)

43. PURIFICATION AND CHARACTERIZATION OF A MAJOR CELL SURFACE GLYCOPROTEIN IN ZAJDELA ASCITES HEPATOMA CELLS WHICH DISPLAYS A POTENT CONCANAVALIN A RECEPTOR ACTIVITY.

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J Cell Biochem; 18(2):245-260 1982

The isolation and characterization of a major cell glycoprotein from Zajdela hepatoma ascites (ZHA) cells, which behaves as a Concanavalin A receptor, was studied to determine which role the cell surface proteins might play in the tumor growth in the system. Effects of trypsin treatment on the amount of radioactivity and protein released from the hepatoma cells are presented. Cell viability after the treatment was greater than 95% and treated cells after 8 hr culture regained the same proliferation capacity as intact cells. Electrophoresis of trypsin digest revealed essentially a single radioactive band strongly stained by periodate-Schiff reagent and a few weakly stained bands. Glycoprotein (GP) II2 appeared homogenous and could be a major constituent released from ZHA cell surface. Carbohydrates represented 32% of the GP II2 molecule, indicating that mol wt of the carbohydrate-free polypeptide could be 72,000 daltons. An indication of GP II2 location was obtained from the cell viability following controlled proteolysis and by specific labeling of sialyl residues with a reagent that theoretically does not enter into the cells. In addition, the metabolic labeling of GP II2 with D-(3H)-glucosamine showed that the GP originated from the cell itself and was not an ascites ligand GP firmly bound to the plasma membrane and hence labeled by the surface labeling procedure. The binding of Concanavalin A on hepatoma cells in the presence of methyl alpha-D-mannoside (0.2 M) or GP II2 (0.01 milliM) was inhibited at 85% and 72%, respectively. Thus, GP II2 proved to be an extremely potent inhibitor of both Concanavalin A binding to the cell surface and its effects on cell proliferation. (55 Refs)

44. CELL SURFACE GLYCOPROTEIN AND ASPARAGINE-LINKED SUGAR CHAIN PATTERNS OF RAT ERYTHROLEUKEMIC CELL LINES.

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Cancer Res; 42(7):2884-2893 1982

In order to elucidate whether chromosomal change or induction of differentiation is correlated with the change of the patterns of cell surface glycoproteins and asparagine-linked sugar chains, glycoproteins and carbohydrates of five rat erythroleukemic cell lines (K1DB, D5A1, K2D, K2DA, and K4D) were studied by sodium dodecyl sulfate:polyacrylamide gel electrophoresis and hydrazinolysis followed by gel chromatography, respectively. The surface glycoprotein patterns of five cell lines were very similar, each containing four glycoproteins with molecular weights of 250,000, 115,000, 80,000, and 28,000, respectively. Only D5A1, which can be induced to erythroid differentiation, had two additional glycoproteins, with molecular weights of 135,000 and 65,000, respectively. All neutral oligosaccharides obtained from plasma membranes of five cell lines by hydrazinolysis were of the high-mannose type, and the acidic oligosaccharides were of the complex type with three different types of outer chains in various combinations: +-NeuAcalpha2 to 6Galbeta1 to 4GlcNAc; Galbeta1 to 3Galbeta1 to 4GlcNAc; and Galbeta1 to 4GlcNAcbeta1 to 3Galbeta1 to 4GlcNAc. An interesting finding was that two sugar chains, NeuAcalpha2 to 6Galbeta1 to 4GlcNAcbeta1 to 2Manalpha1 to 3(Galbeta 1 to 4GlcNAcbeta1 to 2Manalpha1 to 6)Manbeta1 to 4GlcNAcbeta1 to 4(+-Fucalpha1 to 6)GlcNacOT (in which the subscript OT indicates a NaB3H4-reduced oligosaccharide), were found only in surface carbohydrates of D5A1. (Author abstract) (24 Refs)

45. SURFACE GLYCOPROTEIN (S-GP) PATTERNS OF NORMAL AND MALIGNANT LEUKOCYTES (MEETING ABSTRACT)

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12th Workshop on Leukocyte Cultures, February 14-17, 1982, Vienna, Austria Wierner Medizinische Akademie; :A66 1982

The aim of this study was to test the use of surface glycoprotein (S-GP) distribution patterns in the classification of human malignant hemopoietic proliferations. S-GPs were labeled with NaB3H4 after treatment of viable cells with neuraminidase and galactose oxidase. Molecular weight distributions were analyzed by means of PAGE (polacrylamide gel) electrophoresis and fluorography. Samples from more than 200 patients with myeloid and lymphoid proliferations were evaluated. Results were compared with those obtained from purified non-malignant B and T lymphocytes, or monocytes and polymorphs. S-GP patterns of malignant proliferations largely depended upon the derivation of the tumor clones. Marked similarities were observed between blast cells isolated from AML or CML (acute or chronic myeloid leukemia) blast crises and normal polymorphs. Seemingly partial identity was seen between non-Hodgkin lymphoma cells and normal B cells as well as between malignant T cells and normal T lymphocytes. In agreement with earlier reports we thus would state that biochemical analysis of S-GP patterns represents a useful tool in the classification of human leukemias and lymphomas. (no Refs)

46. A NEW MURINE CELL SURFACE DIFFERENTIATION ANTIGEN (LEUGP90) DEFINED BY A RAT MONOCLONAL ANTIBODY: CELLULAR DISTRIBUTION AND BIOCHEMICAL CHARACTERIZATION.

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J Immunol; 128(6):2664-2669 1982

Monoclonal antibodies to murine lymphocyte differentiation antigens were generated by fusing the mouse myeloma cell line X63-Ag8.653 with spleen cells derived from Lewis rats hyperimmunized with lymphoid cells from nude (C57BL/6) mice.

One of these antibodies - designated 3MB1-recognizes a previously undescribed cell surface antigen. This antigen is expressed on 62% of spleen cells, 9% of thymus cells, and 98% of peritoneal macrophages. Virtually all LPS- and Con A-induced lymphoblasts carry this newly found antigen. The 3MB1 determinant is limited in its expression to leukocytes. It is not found in other tissues such as brain, liver, kidney, or on erythrocytes. Biochemical analysis reveals that the 3MB1 target antigen consists of a single chain glycoprotein with an approx mw of 90,000. (Author abstract) (43 Refs)

47. THE SURFACE PROTEINS OF HUMAN ASTROCYTOMAS IN CULTURE (MEETING ABSTRACT)

Sherbet GV, Wildridge M, Kalbag RM
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Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

Cell cultures were initiated from human astrocytomas. Surface proteins of subconfluent cultures were labelled by lactoperoxidase-catalyzed radio-iodination, and separated by electrophoresis on 6.0% cylindrical polyacrylamide gels. Normal glial and astrocytoma cells were found to possess a common surface protein pattern, but there were significant quantitative changes in the expression of certain protein components of the astrocytomas as compared with normal glial cells. A component of mol wt approx 225K which appears, by criteria of electrophoretic behavior and immunological reactivity with mono-specific anti-fibronectin antibodies, to be fibronectin was reduced by between 15-70% in 6/8 astrocytomas. However, receptors for fibronectin could be detected on the surface of the cells. Another component(s) of average mol wt of 148K appeared to be amplified by a factor of 1.6-3.5 in all astrocytomas. Surface components of mol wt less than 75K also incorporated more radioiodine than did the corresponding external proteins of normal glial cells. It is suggested that some of these changes, especially in the 225K and 148K external proteins, may be associated with the malignant state. (no Refs)

48. THE ROLE OF CELL SURFACE PROTEINS IN THE INDUCTION OF HISTOGENETIC DIFFERENTIATIVE RESPONSES BY SOME HUMAN BREAST TUMOURS AND HAMSTER TUMOURS IMPLANTED INTO CHICK EMBRYOS.

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Exp Cell Biol; 50(3):169-179 1982

Cells of human breast tumors and fibrocystic hyperplasia grown in culture, and three hamster tumors were implanted between the cell layers of 18-hr-old chick blastoderm. Their ability to induce histogenetic responses in the ectodermal and endodermal embryonic tissues was investigated. The surface proteins of these tumor cells were labeled by lactoperoxidase-catalyzed radioiodination. It is shown that the ability to induce the histogenetic effects may be related to the expression of 265K (K = 10(3) daltons) and 233K proteins on the surface of human tumor cells and of 115 K proteins on the hamster tumor cells. The antiproteinase, aprotinin, inhibits the induction of the histogenetic responses by and apparently also prevents the deletion of 115K proteins from the hamster tumor cells. It is therefore suggested that cell surface proteins are involved in the complex processes of interaction between embryonic and tumor cells and in the recognition by the embryonic cells of the tumor cells implanted into their midst. (Author abstract) (23 Refs)

49. METASTATIC PROPERTIES AND CELL SURFACE BIOCHEMISTRY OF CLONES ISOLATED FROM AN HSV-2 TRANSFORMED HAMSTER CELL LINE (MEETING ABSTRACT)

Walker JR, Rees RC, Potter CW
Clinical Research Laboratory, Weston Park Hospital, Sheffield, England
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

After resection of subcutaneous tumor derived from the inoculation of the HSV-2-333-2-26 cell line into Syrian golden hamsters, a small proportion of animals developed metastatic lesions at secondary sites. Selection of variants by subcutaneous re-implantation of lung foci has produced two sublines with a markedly increased metastatic potential when compared with the parent line. In addition, numerous differences in cell surface glycoproteins labelled by the galactose oxidase - tritiated sodium borohydride technique have been reported. The study of these cell lines has been extended by the repeated subcutaneous transplantation of lung and kidney foci in order to produce lines of different organ propensity. The parent, poorly metastatic cell line has been cloned in vitro and a range of clones of different malignancy has been obtained. Preliminary studies have suggested that some cell surface glycoprotein components also differ between the cell lines. (1 Ref)

50. DIRECT MODIFICATION OF THE MEMBRANE ADENYLATE CYCLASE SYSTEM BY ISLET-ACTIVATING PROTEIN DUE TO ADP-RIBOSYLATION OF A MEMBRANE PROTEIN.

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Proc Natl Acad Sci USA; 79(10,part1):3129-3133 1982

GTP and isoproterenol activation of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing) EC 4.6.1.1] in washed membranes prepared from C6 glioma cells was enhanced by incubation with islet-activating protein, one of the pertussis toxins, if the incubation mixture was supplemented with NAD and ATP. The action of the protein was observed immediately after its addition and increased progressively in magnitude as the protein concentration or the incubation time increased. There was simultaneous incorporation of radioactivity from the ADP-ribose moiety of variously labeled NAD into the membrane protein with a mol wt of 41,000. We conclude that islet-activating protein enhances receptor-mediated GTP-induced activation of membrane adenylate cyclase as a result of ADP-ribosylation of a membrane protein, probably one of the components of the receptor-adenylate cyclase system. (Author abstract) (21 Refs)

51. EARLY AND LATE CATHEPSIN D-DERIVED FRAGMENTS OF FIBRONECTIN CONTAINING THE C-TERMINAL INTERCHAIN DISULFIDE CROSS-LINK.

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Hoppe Seylers Z Physiol Chem; 363(4):351-364 1982

Fibronectin consists of two very similar subunits connected by disulfide bonds close to their C-terminal ends. After short digestion with cathepsin D a fragment containing an intact subunit linked to a C-terminal piece of the other chain was isolated. Both possible combinations were realized. The remnant chain, removed by reduction, had a relative molecular mass (Mr) of 65,000 or 75,000, respectively, whether it originated from the shorter or the longer fibronectin subunit. After prolonged cathepsin D digestion another disulfide-linked fragment of Mr 90,000 containing peptide chains of Mr 65,000 and Mr 36,000 was found. The same fragment also formed during prolonged digestion of a previously described cathepsin D-derived heparin-binding piece of Mr 140,000 which consisted of disulfide-linked chains of Mr 65,000 and Mr 75,000. The cut-off material emerged as a mixture of two similar heparin-binding peptides of Mr 35,000 and Mr 37,000. The residual disulfide-linked peptide of Mr 90,000 was retained by heparin-Sepharose, too, indicating that the non-degraded chain of Mr 65,000 also contained a heparin-binding site. Plasminolysis of the Mr 140,000 fragment first liberated the two individual chains and subsequently cleaved the longer one into two domains of nearly equal size. A rather basic one (pI 8.4-8.8) with low cystine content bound to heparin-Sepharose, while the other cystine-rich and more acidic domain (pI 5.0-5.8) was not retained. The shorter peptide chain, also showing affinity to heparin, was attacked by plasmin rather slowly. It, however, also appeared to be composed of two differently charged regions as it had an isoelectric point in the neutral range (pI 6.4-6.8) in spite of its heparin-binding properties. In the longer chain the heparin-binding domain was located in a distal position to the C-terminal disulfide link. The same is assumed for the shorter chain by reason of homology. (Author abstract) (18 Refs)

52. PROPERTIES OF LYMPHOCYTE 5'NUCLEOTIDASE IN NORMAL SUBJECTS AND PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

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Leuk Res; 6(2):203-210 1982

Heterogeneity of 5'nucleotidase (5NT) was studied in normal and in chronic lymphocytic leukemia (CLL) lymphocytes. 5NT reaction velocity was proportional to the incubation time for up to 2 hr to the amount of protein over a concentration ranging from 1-52 ug/assay. The effect of various substrates on the reaction rate of plasma membrane 5NT obtained from normal and CLL lymphocytes is shown. When lymphocyte membranes from 5NT negative CLL donors were assayed, no activity with any of the substrates was observed even when the incubation period was extended to 5 hr. Inosine monophosphate was compared to AMP as a substrate using nonhomogenized cells and subcellular fractions from normal and CLL lymphocytes. AMP was consistently a better substrate for the ecto-5NT. A very wide but reproducible range of Km values, from 19-210 uM was found with nonhomogenized lymphocytes while a much lower Km value with a narrow range was observed with the plasma membranes. An unexpected relationship was observed between Km values and the specific activity of the lymphocytes. Lymphocyte preparations which had higher specific activity also had higher Km values for AMP. Dialysis of lymphocyte homogenates overnight against Tris-dithiothreitol buffer resulted in a marked decrease in Pi levels but had no effect on the Km. The addition of Pi to a final concentration of 1 milliM did not affect the Km. Studies of heat stability showed that the enzyme in CLL and in normal lymphocytes was relatively stable at 54 C. (20 Refs)

53. IMMUNOFLUORESCENT DETECTION OF ERYTHROCYTE SIALOGLYCOPROTEIN ANTIGENS ON MURINE ERYTHROID CELLS.

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J Cell Biol; 93(3):591-603 1982

A sialoglycoprotein fraction isolated from murine (DBA/2) erythrocytic ghosts was used to raise antibodies in rabbits. By immune-IgG (serum)-[125I]protein A overlays, the antibodies were found to react positively with the four sialoglycoprotein monomers (gp-2.1, gp-2.2, gp-3.1 and gp-3.2) of the original fraction, with the sialoglycoproteins detected in erythrocytic ghosts (gp-2.1 and gp-3.1), with a diffuse component (probably a macroglycolipid) trailing around gp-3.1 in SDS polyacrylamide gel electrophoretograms of solubilized ghosts, and with a minor sialoglycoprotein hidden under this trail. IgG's isolated from immune and nonimmune rabbit sera were conjugated to tetramethylrhodamine isothiocyanate and used to survey, by fluorescence microscopy, the distribution of the cognate antigens on the three different erythroid lines known to succeed each other during the life span of the mouse. In the peripheral blood of the adult, the antibodies recognized only mature erythrocytes; they did not crossreact with either platelets, monocytes, or different types of granulocytes. In the spleen of adult anemic mice, the antibodies reacted weakly with proerythroblasts and strongly with all types of erythroblasts. In enucleating erythroblasts, antigens were preferentially segregated on the cell membrane of the nascent reticulocyte. In the 10-day-old embryo, antigens were already present on the primitive nucleated erythrocytes (produced by the blood islets of the yolk sac), and in the 14-d fetus they were found on all hepatic erythroblasts and derived non-nucleated erythrocytes. A positive immunoreaction was also obtained on Friend erythroleukemic cells, before or after induction by dimethyl sulfoxide. Nonimmune serum, or nonimmune IgGs gave negative reactions in all cases. The antibodies were species-specific: they did not crossreact with either human or rat erythrocytes. (Author abstract) (70 Refs)

54. FLUORESCEINATED LECTINS AS PROBE FOR CELL SURFACE CHANGES ASSOCIATED WITH LYMPHOCYTE TRANSFORMATION.

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Am J Hematol; 12(3):227-232 1982

Lectins are plant-derived proteins with specific affinities for simple or complex sugars and are potentially useful tools for investigation of the carbohydrate structure of the cell surface. A panel of fluoresceinated lectins was used to study the cell surface changes associated with lymphocyte transformation. The major determinants detected on peripheral blood lymphocytes were alphaD-mannose,beta-D-galactose and N-acetyl-glucosamine. Phytohemagglutinin transformation was associated with increased binding of lectins specific for N-acetyl-galactosamine. MOLT-4, a T-lymphoblast cell line in continuous culture, was associated with increased binding of lectins specific for N-acetyl-galactosamine and L-fucose. Application of fluoresceinated lectins may be a useful tool in the classification of lymphoid cells. (Author abstract) (10 Refs)

55. TWO-DIMENSIONAL GEL ELECTROPHORESIS OF MEMBRANE PROTEINS FROM THE R3327 PROSTATE ADENOCARCINOMA.

Kozlovskis PL, Claflin AJ, Fletcher MA, McKinney EC, Rubin RW
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Cancer Res; 42(7):2748-2756 1982

The Dunning rat prostate adenocarcinoma (R3327) is a reliable model that shares many similarities with the human tumor. Two sublines of the tumor, G and H, represent opposite extremes in histology and growth rate. Purified membrane fractions from G and H solid tumors were isolated by sucrose gradient. Tumor and normal prostate membrane proteins were labeled with 125I, incubated with G or H antisera, and precipitated by absorption of antibody-antigen complexes to staphylococcal Protein A. Proteins were resolubilized and electrophoresed on two-dimensional gels, and the gels were autoradiographed. A total of eight labeled proteins were precipitated from the G and H tumors in the presence of G antisera. Of these, seven were homologous. One high-mol-wt protein (Protein b) present on the G tumor was absent from the H tumor. The H tumor contained high-mol-wt protein (i) that was not found on the G tumor or on normal prostate. Normal prostate revealed a pattern similar to the G tumor except that Protein b appeared to be quantitatively reduced. Precipitation in the presence of H antisera showed similar patterns except that Protein b was not detected in the G tumor and was greatly reduced in the normal prostate. Therefore, despite variable growth characteristics, there were few changes in membrane proteins between the solid tumors and between the tumors and normal prostate. Iodination of surface proteins of cultured cells from normal prostate and the G and H sublines also showed a high degree of homology. No consistent differences between cultured cell lines were noted. (Author abstract) (27 Refs)

56. MOLECULAR CHARACTERIZATION OF PREIMPLANTATION EMBRYONIC SURFACE ANTIGENS WITH AN ANTITERATOCARCINOMA SERUM (MEETING ABSTRACT)

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Anat Rec; 202(3):12A 1982

Cell surface antigens expressed by preimplantation mouse embryos and teratocarcinomas are studied using a rabbit antiserum (A-N1) against clonally derived nullipotential mouse teratocarcinoma stem cells (Nulli-SCC-1). Indirect immunofluorescen studies indicate that following absorption with adult mouse tissue, A-N1 recognizes surface antigens on all preimplantation stage mouse embryos including blastocyst trophectoderm and inner cell mass. N1 antigens are also found on the female germ line but not male. Development is inhibited when mouse embryos are cultured in the presence of A-N1 serum suggesting a possible developmental role for the antigens detected. In vivo absorption of A-N1 in adult male or female mice does not remove all A-N1 activity suggesting one or more antigens detected are embryo specific. Using Staphylococcus aureus mediated immunoprecipitation followed by 1-D and 2-D electrophoresis, we have identified 7 protein or glycoprotein antigens which are specifically precipitated from embryos and teratocarcinoma cells (TC) with A-N1. Differential absorption of A-N1 with whole live TC cells vs detergent lysate of TC cells has permitted the differentiation between cell surface and cytoplasmic antigens. Four of the 7 precipitated are cell surface antigens with approximate molecular weights of 16,000, 18,000, 37,000 and 60,000 and appear on both mouse blastocysts and nullipotential TC cells. Precipitation with in vivo absorbed A-N1 is being used to determine which of these antigens is embryo specific. (1 Ref)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

57. THE LOCATION AND SYNTHESIS OF TRANSFERRIN IN MOUSE EMBRYOS AND TERATOCARCINOMA CELLS.

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Dev Biol; 91(2):227-234 1982

In early postimplantation mouse development, transferrin synthesis appears to be a marker of visceral endoderm cell types. Transferrin was identified using immunoperoxidase staining, in the proximal (visceral) endoderm of the 6th-day egg cylinder, in some tissues at later stages, and in the visceral yolk sac (VYS) at all stages examined. Since the location of a plasma protein does not necessarily indicate its site of synthesis, the incorporation of labeled amino acids into transferrin was studied. Synthesis could be detected in egg cylinders on the 7th day of gestation onwards and in the VYS at all stages. However, although endoderm was the likely tissue source, its ability to synthesize transferrin after its isolation from the embryo was either much reduced or absent. The data are suggestive of a modulating influence by mesoderm and other cell types on transferrin synthesis in visceral endoderm cells. Three types of endoderm-like cells which are produced by teratocarcinoma embryonal carcinoma (EC) cells were analyzed for transferrin synthesis to assess possible parallels with the embryo. Embryoid bodies from PSA1 EC cells contained some outer endoderm cells which stained for transferrin and others which did not. The endoderm line PSA5E but not PYS-2 synthesized transferrin. The third type of endoderm-like cell (END cells) synthesized very little (OC15S1) or no (PC13 clone 5) transferrin. The conclusion that PSA5E, OC15 END, and some differentiated PSA1 cells have visceral endoderm-like character while PYS-2 reflects parietal endoderm phenotype is in agreement with published data. (Author abstract) (41 Refs)

58. MODULATION OF SYNTHESIS OF SPECIFIC PROTEINS IN ENDOTHELIAL CELLS BY COPPER, CADMIUM, AND DISULFIRAM. AN EARLY RESPONSE TO AN ANGIOGENIC INDUCER OF CELL MIGRATION.

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J Cell Physiol; 111(2):207-212 1982

The effects of copper, cadmium, and disulfiram on the synthesis of proteins by two lines of bovine endothelial cells were investigated. Induction of protein synthesis by these agents was observed in one of the cell lines, indicating that fibroblasts are not the only cell types. Disulfiram inhibited the expression of several large mol wt proteins, including secreted fibronectin and possibly laminin. (17 Refs)

59. IS TRANSFORMATION ASSOCIATED WITH AN INCREASED ERROR FREQUENCY IN MAMMALIAN CELLS?

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J Biol Chem; 257(11):5977-5979 1982

Acute starvation of mammalian cells for amino acids results in translational errors that may be detected by two-dimensional polyacrylamide gel electrophoresis. Using this as an assay for error frequency in mammalian cells, we investigated the hypothesis that neoplastic transformation was associated with an increased error frequency which in turn leads to an increased mutation rate and a decreased efficiency of regulatory controls (phenomena of tumor progression). Although we found that transformation was not always associated with an increased level of mistranslation we showed that SV40 transformation increased the level of translational errors in all cell types tested. (Author abstract) (27 Refs)

60. GAMMA HEAVY CHAIN DISEASE IN MAN: CDNA SEQUENCE SUPPORTS PARTIAL GENE DELETION MODEL.

Alexander A, Steinmetz M, Barritault D, Frangione B, Franklin EC, Hood L, Buxbaum JN
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Proc Natl Acad Sci USA; 79(10,part1):3260-3264 1982

Human gamma heavy chain disease (HCD) is characterized by the presence in serum of a short monoclonal Ig gamma chain unattached to light chains. Although most HCD proteins have internal deletions, in some the defect is NH2-terminal. The OMM gamma3 HCD serum protein is of the latter type, having undergone an extensive NH2-terminal deletion with a sequence starting within the hinge. A cell line synthesizing the OMM protein has enabled us to study the biogenesis of the abnormal molecule. In vitro translation of isolated mRNA yields a protein containing a hydrophobic NH2-terminal leader sequence. In the intact cell, the precursor molecule is processed normally to yield a protein with an NH2-terminal sequence homologous to the beginning of the variable (V) region. The nucleotide sequence of cDNA prepared from the OMM mRNA encodes a 19-amino acid leader followed by the first 15 residues of the V region. An extensive internal deletion encompasses the remainder of the V and the entire CH1 domain. Immediately following the short V region, there is information in the cDNA for the entire normal hinge. The primary synthetic product is thus an internally deleted molecule that undergoes postsynthetic degradation to yield the NH2-terminally deleted serum protein. The structure of the OMM mRNA suggests that the protein abnormality results from a partial gene deletion rather than defective splicing. (Author abstract) (45 Refs)

61. MOLECULAR CLONING OF HUMAN IMMUNE INTERFERON CDNA AND ITS EXPRESSION IN EUKARYOTIC CELLS.

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Nucleic Acids Res; 10(8):2487-2501 1982

Starting with mRNA derived from Staphylococcal enterotoxin A induced human splenocytes, dsDNA was synthesized and inserted into the unique BamHI site of the eukaryotic expression vector pSV529. A recombinant plasmid containing human immune interferon (IFN-gamma) cDNA was identified by hybridization of plasmid inserted DNA bound onto nitrocellulose filters with mRNA derived from SEA-induced splenocytes, translation of the eluted RNA in Xenopus laevis oocytes and assaying from IFN activity. Plasmids containing the entire human IFN-gamma cDNA sequence were identified by colony hybridization and were sequenced. A unique coding region was identified which predicted a protein of 166 amino acids, the 20 N-terminal amino acids of which presumably represent a signal peptide. After transfection of monkey cells with plasmid DNA isolated from one of the recombinant clones (pHIIF-SV-gamma1), IFN was excreted into the culture medium. This IFN was not distinguishable from human IFN-gamma by serological criteria or by cell target species specificity. (Author abstract) (53 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

62. INDUCTION OF ALKALINE PHOSPHATASE AND THE GLYCOPROTEIN HORMONE ALPHA SUBUNIT IN HELA CELLS BY INHIBITORS OF DNA POLYMERASE.

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Arch Biochem Biophys; 216(1):234-246 1982

Effects of two eukaryotic DNA polymerase inhibitors, aphidicolin (ApH) and phosphonoformic acid (PFA) on synthesis of placental alkaline phosphatase (PAP) and of the glycoprotein hormone alpha subunit in Hella cells were investigated. Both ApH (1.0-5.0 ug/ml) and PFA (1.2 or 3.8 milliM) inhibited DNA synthesis almost completely (82-93%), even at the lowest concentrations tested. Both inhibitors were good inducers of both PAP and the glycoprotein hormone alpha subunit. PAP activity was enhanced 60-100% by ApH at 1 ug/ml, and alpha subunit levels were increased 5-fold at an ApH concentration of 5 ug/ml. PAP activity showed a 7-to 8-fold increase and alpha subunit and a 2-to 3-fold increase as PFA concentration was increased to 3 milliM. Partial characterization of induced alpha subunit and PAP activity suggested that they are similar to the uninduced proteins. ApH (2.ug/ml) and PFA (2.5 milliM) acted synergistically with butyrate (3 milliM) but not with hydroxyurea (1 milliM). The fact that the concentration of ApH required to produce a half-maximal induction of the alpha subunit (15 ug/ml) was of an order of magnitude higher than the concentration required to inhibit DNA synthesis by 50% (0.1 ug/ml) makes it unlikely that a direct relation exists between protein induction and DNA synthesis inhibition. (53 Refs)

63. EFFECT OF TUNICAMYCIN ON INSULIN BINDING AND ON PROTEOGLYCAN SYNTHESIS AND DISTRIBUTION IN SWARM RAT CHONDROSARCOMA CELL CULTURES.

Stevens RL, Schwartz LB, Austen KF, Lohmander LS, Kimura JH
Dept. Medicine, Harvard Medical Sch., Boston, MA, 02115
J Biol Chem; 257(10):5745-5750 1982

Tunicamycin, an inhibitor of dolichol-diphospho-N-acetylglucosamine formation and hence an inhibitor of N-linked oligosaccharide biosynthesis, suppressed total proteoglycan synthesis by Swarm rat chondrosarcoma chondrocytes without affecting the size of the proteoglycan molecule, its secretion from the cell, or its ability to be retained in the extracellular matrix. In addition, tunicamycin did not substantially alter the ability of the chondrocytes to polymerize glycosaminoglycan onto an exogenous beta-D-xyloside acceptor. A secondary effect of tunicamycin suppression of proteoglycan synthesis was that a lesser amount of newly synthesized proteoglycan diffused from the extracellular matrix into the culture medium. The ability of exogenous hyaluronic acid and proteoglycan to increase the percentage of newly synthesized 35S-proteoglycan in the medium in tunicamycin-treated cultures indicates that matrix retention of 35S-proteoglycan is related to the total extracellular uronic acid content rather than to the presence or absence of mannose oligosaccharides bound to the proteoglycan molecule. These noncytotoxic concentrations of tunicamycin (33-333 ng/ml) decreased [3H]mannose incorporation to the same extent that they decreased total [35S]sulfate and [3H]serine incorporation, and caused the chondrocyte to synthesize and secrete a species of beta-hexosaminidase that was mannose-deficient as assessed by its failure to bind to concanavalin A. The additional finding of decreased insulin binding to tunicamycin-treated chondrosarcoma chondrocytes suggested that the inhibition of proteoglycan synthesis was due to diminution of receptors which respond to stimulatory hormones. (Author abstract) (24 Refs)

64. PHYSIOPATHOLOGICAL AND BIOCHEMICAL CHANGES IN DOGS WITH VARIOUS CLINICAL VARIANTS OF INHALATION POISONING WITH 239PU AND 241AM NITRATES.

Kalmykova ZI, Tokarskaia ZB, Buldakov LA, Nifatov AP, Vedeneev VS
No affiliation given
Radiobiologiia; 22(2):209-214 1982

Effects of inhalation exposure to nitrates of 239Pu polymer and 241 Am monomer were studied in dogs. Both radionuclides produced progressive respiratory insufficiency, pneumonia, and pneumosclerosis. Clinical manifestations of respiratory insufficiency after exposure to 239Pu were more pronounced than those after exposure to 241Am. Analysis of blood proteins showed hyperproteinemia after exposure to 239Pu and hypoproteinemia after exposure to 241Am. 239Pu induced early and stable increase of a number of carbohydrate components of glycoproteins, compared with an increase of seromucoids alone induced by 241Am. Activities of SGOT and SGPT were decreased after exposure to 239Pu and increased after exposure to 241Am. Activity of sorbol dehydrogenase in dogs exposed to 241Am was lower than that in dogs exposed to 239Pu. (16 Refs) (In RUS)

65. METABOLIC CHANGES AFTER EXPOSURE TO RADIONUCLIDES.

Kalistratova VS
No affiliation given
Med Radiol (Mosk); 27(4):62-71 1982

Literature data on the effects of radionuclides on lipid, carbohydrate and protein metabolism are reviewed. Studies of lipid metabolism showed that 132I induced significant increase of serum cholesterin level. Exposure to 90Sr, 137Cs, and 131I produced a decrease of serum cholesterol level and decrease of activity of serum cholinesterase (CE). Inhalation exposure to 239Pu increased the phospholipid levels in the lung tissue. Impairment of carbohydrate metabolism was observed after exposure to 210Po, 32P, 90Sr, and 131I. The changes in carbohydrate metabolism included decrease of glycogen level in the liver and in the muscles, and transient hyperglycemia. Studies of the protein metabolism showed that exposure to 239Pu, 90Sr, 228Th, 228Ra, 210Po, 237Np, 137Cs, 131I, and 32P produced marked increase of the activities of alkaline phosphatase (AP) and transaminases, decrease of CE activity, and increase of serum proteins levels. The increase of AP activity was correlated with the yield of radiation-induced osteosarcomas. (65 Refs) (In RUS)

66. EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON RNA POLYMERASE I INDUCTION IN RAT LIVER (MEETING ABSTRACT)

Potter CL, Sipes IG, Russell DH
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Toxicologist; 2(1):146 1982

Previously, we have reported that pretreatment of rats with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits the extent of hepatic induction of ornithine decarboxylase, the rate limiting enzyme in biosynthesis of the polyamines (putrescine, spermidine and spermine). Since increased hepatic ornithine decarboxylase activity has been positively correlated to a subsequent increase in RNA polymerase I activity, we examined the effects of TCDD on RNA polymerase I induction. Male Sprague-Dawley rats (100-150 g) received a single ip injection of 5 ug/kg TCDD in acetone:corn oil (1:17). Hepatic RNA polymerase I activity was subsequently stimulated 25-50% by partial hepatectomy or by administration of dexamethasone (0.5 mg/kg) in normal saline. At 5 hr or 16 hr after partial hepatectomy, or 4 hr after dexamethasone, times at which TCDD has been shown to strongly inhibit ornithine decarboxylase induction, animals pretreated one week earlier with TCDD exhibited RNA polymerase I activity 20-50% lower than controls: ie TCDD-treated rats exhibited 0-50% as much stimulation of RNA polymerase I activity as did the controls. In unstimulated liver, RNA polymerase I activity, as well as protein DNA, RNA and spermine levels, remained essentially the same in control and TCDD-treated rats one week after treatment. However, TCDD-treated rats had significantly lower putrescine and spermidine concentrations. (no Refs)

DIETARY AND NUTRITIONAL EFFECTS

67. INFLUENCE OF DIETARY PROTEIN LEVELS ON SURVIVAL OF RATS FOLLOWING KIDNEY IRRADIATION.

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Int J Radiat Oncol Biol Phys; 8(5):931-935 1982

Influence of dietary protein levels on rats survival following kidney irradiation was studied in young adult male Sprague-Dawley unilaterally nephrectomized rats. The remaining kidney was exteriorized and exposed to 14 Gray units x-rays. The survival curves for non-irradiated control groups are presented. The major cause of death amount these animals was pneumonia. Survival following exposure to x-rays was strongly correlated to diet. Median survival for the 50% protein animals was 53 days in one replicate and 65 days in the other. In contrast, the median survival times of the two 20% protein diet replicates were 95 days and 100 days. The ketone and glucose levels were very low. Typical pH levels were 6 or 7, with no obvious correlation existing between animals with not typical urine pH and their treatment. With time, blood did begin appearing in some urines. A blood value of +2 in the urine was a diagnostic impending death of the animal; generally within a wk to 10 days after being detected in the urine. Food consumption was measured for 2 to 4 animals from each tap water group for 9 consecutive days. A slight difference in food consumption among the three levels of dietary protein, with 14 Gray units animals on 4% protein diet consuming 12.6 g/day, those on 20% diet consuming 14.5 g/day, and those on 50% diet consuming 13.5 g/day was observed. The difference was statistically significant. Urine production was elevated in the groups that received 0.9% NaCl drinking water as opposed to tap water. (21 Refs)

68. ASSESSMENT OF NUTRITIONAL PROTEINS DURING THE PARENTERAL NUTRITION OF CANCER PATIENTS.

Bourry J, Milano G, Caldani C, Schneider M
Centre Antoine Lacassagne, Nice, Cedex, France
Ann Clin Lab Sci; 12(3):158-162 1982

Albumin, prealbumin, transferrin, and retinol-binding protein (RBP) concentrations were measured in sera of 39 patients (22 men, 17 women; mean age 64.6 yr) hospitalized for cancer or cancer related complications, and the variations in protein values during parenteral nutrition (PN) were recorded. Two patient groups were defined: G1, which comprised 22 patients who lived at least 1 mo after PN or within the mo after PN had been stopped and G2, which comprised 17 patients who died during PN or within the mo after PN was stopped. Protein concentrations were lower in G2 than in G1, except RBP in G2, although the differences were not significant. During refeeding, prealbumin was the only protein that increased in concentration; all other nutritional parameters decreased. Prealbumin was the parameter showing the greatest change from before the beginning of PN to after 3 wk in G1. G2 patients showed similar increases in prealbumin initially, but values declined after the third wk for this group; the difference in pattern between the two groups was significant. All protein values measured were considerably lower in cancer patients than in controls, confirming the value of these indices of malnourishment when applied to cancer patients. The drop in prealbumin after 2 wk of PN seems to indicate a fatal outcome. (21 Refs)

69. INTERMITTENT FASTING AND RE-FEEDING EFFECTS ON LEWIS LUNG TUMOR AND HOST WEIGHTS (MEETING ABSTRACT)

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Med Pediatr Oncol; 10(1):100 1982

Restriction of food intake has been known to alter the growth of specific tumors, but little has been reported with regard to the effects of restriction on the host. The purpose of the present study was to examine the effects on tumor and host weights as a function of two intermittent fasting and re-feeding schedules. Thirty C57/Bl mice were inoculated in the flank with 5x10(5) viable Lewis Lung cells, the animals were separated into three groups of ten animals each, all animals subsequently were fed an amino acid defined diet and killed after sixteen days. Group I (control) was fed ad-libitum for the entire period; Group II was pair fed to Group I and maintained on a 33/15 hr fed/fast schedule; and Group III was maintained on a 24/24 hr fed/fast schedule. The tumor weight (TW) and the tumor to host weight ratio (T/H) of Group II were reduced to 69% of the control values even though they ingested the same quantity of diet over the experimental period. Since there was no significant difference in host weight between those two groups, this feeding schedule served to reduce tumor growth without compromise to the host weight resulting in an improved ratio of tumor to host weight. Group III consumed less food (74% of control) than the other two groups which resulted in a decrease in both tumor weight (50% of control) and host weight, but the tumor to host ratio was not significantly reduced below that found for Group II. Thus, the stringent fasting regimen of Group II showed no greater overall benefit with regard to tumor vs host response that was achieved by the less restrictive feeding schedule of Group II. (no Refs)

70. PROTEIN METABOLISM AND TUMOR GROWTH (MEETING ABSTRACT)

Steinberg JJ, Stein TP, Presti M, Miller EE, Mullen JL
Department of Pathology, New York University School of Medicine, New York, NY
71st Annual Meeting of the International Academy of Pathology (United States-Canadian Division), March 1-5, 1982, Boston, Massachusetts. International Academy of Pathology 95 pp., 1982.

To study the affects of tumor growth on protein metabolism, branch chain amino acids (BCAA), 15 mixed amino acids (MAA), or alanine (ALA) was infused into Lewis-Wistar rats (192+/-gm) after tumor inoculation (3.0 x 10(7) cell count) and 5 days of tumor growth (1.9 cu cm). Fifty-one rats underwent superior vena cava canulation and were assigned to 6 days of one of three isocaloric, isonitrogenous, and isovoloemic diets (168 kcal per kg per day, 1.08 gm of nitrogen per kg per day), differing only in nitrogen source: BCAA, MAA, or ALA. Fourteen rats remained as rat show controls. Protein flux, synthesis, and catabolism (as milligrams of nitrogen per day) were calculated from urine N15 enrichment after glycine-N15 infusion. At sacrifice, BCAA and ALA host weight decline were surprisingly equivalent to rat chow and MAA groups, althout MAA better maintains 6-day cumulative nitrogen balance than BCAA and ALA. Tumor volume exponential growth, doubling time and tumor:host weight ratios were equivalent. BCAA do not affect tumor growth significantly from other dietary regimens. BCAA are less effective than MAA but more effective than ALA in maintaining host nitrogen. (no Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

71. REPLACEMENT OF NATURAL POLYAMINES BY CADAVERINE AND ITS AMINOPROPYL DERIVATIVES IN EHRLICH ASCITES CARCINOMA CELLS.

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Biochem Biophys Res Commun; 106(2):291-297 1982

The replacement of the polyamines spermidine and putrescine by cadaverine in Ehrlich ascites carcinoma cells was found to be compatible with continued growth in these cells, although cell division was slowed and the changes typical of polyamine-depletion were expressed. Cells were polyamine-depleted by culture in the presence of 5 milliM difluoromethyl ornithine (DFMO) and 0.005 milliM cadaverine. Under such conditions the natural polyamines were almost completely replaced by cadaverine and its aminopropyl derivatives, mainly N-(3-aminopropyl)cadaverine. While the synthesis of RNA and DNA was decreased by only about 20% in polyamine-depleted, cadaverine containing cells, the incorporation of labeled leucine into protein was depressed by 50%. Tumor cells grown in the presence of DFMO and cadaverine exhibited ornithine decarboxylase activity 10 x higher than untreated cells. The activity of adenosylmethionine decarboxylase was also greatly elevated in cells exposed to DFMO and cadaverine, a phenomenon typical of polyamine-depleted cells. Cadaverine containing cells fully retained the enhanced uptake of methylglyoxal bis(guanylhydrazone), another characteristic of polyamine-depleted cells. (22 Refs)

72. INTERFERON INDUCTION OF POLYAMINE-DEPENDENT PROTEIN KINASE ACTIVITY IN EHRLICH ASCITES TUMOR CELLS.

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Biochem Biophys Res Commun; 106(2):305-311 1982

The finding of an interferon-induced protein kinase activity in Ehrlich ascites tumor cells which is activated by the polyamines spermidine and spermine is reported. The cytoplasmic fraction of interferon-treated cells demonstrated an enhanced level of protein kinase activity compared to controls, and the activity was enhanced 2.8x by the polyamines or by poly(I).poly(C). Simultaneous additions of the polyamines and double stranded RNA produced a 4x stimulation. The nuclear fraction of interferon-induced cells also exhibited a marked enhancement of protein kinase activity, with a pattern similar to that observed in the cytoplasmic fraction. Interferon elicited a 33x induction of polyamine-dependent protein kinase activity in the nucleus. Combined additions of the polyamines and double stranded RNA evoked no significantly different response than polyamines alone, suggesting that a single protein kinase may be activated both by a spermidine/spermine mixture and by double stranded RNA. Partial purification of cell extracts on poly(I).poly(C)-agarose or by fractionation on phosphocellulose columns supported this suggestion. (16 Refs)

73. IDENTIFICATION OF TWO INHIBITORS OF HEPATOMA POLYAMINE RESPONSIVE PROTEIN KINASE SYSTEM (MEETING ABSTRACT)

Morishita Y, Sahai A, Akogyeram C, Liederman L, Oka T, Criss WE
Cancer Center, Washington, DC, 20059
Med Pediatr Oncol; 10(1):102 1982

A polyamine responsive kinase (PPK) system was first identified and purified from the cytosol of a series of fast growing Morris hepatomas. The native enzyme was resolved into a catalytic component (MW 210,000) and several substrates or phosphate acceptor proteins (all less than MW 100,000). This report presents data that the hepatoma PPK systems also contains two dialyzable inhibitor molecules. The inhibitors were identified and purified through two different purification schemes: 1) DEAE (diethylaminoethyl) cellulose, Sephacryl S-300, Sephadex G-25, and phosphocellulose column chromatographies; 2) extensive dialysis using a 50,000 MW exclusion dialysis membrane, lypholyzation of dialyzing medium, and Sephadex G-50 column chromatography. Inhibitor I had a molecular weight of 500-600 and inhibited both the basal and polyamine stimulatable activities when the dialyzed enzyme was evaluated. Inhibitor II had molecular weight near 1,800 and also inhibited both basal and stimulatable activities when the dialyzed enzyme was evaluated. Both inhibitors were heat stable and appear to interact with the substrate binding site on the intact enzyme. They are bound to the enzyme in vivo, as the entire system, catalytic unit, substrates, and inhibitors copurify in the initial purification stages. (1 Ref)

OTHER RELATED STUDIES

74. CONTRASTS IN TRANSPORT SYSTEMS FOR ANIONIC AMINO ACIDS IN HEPATOCYTES AND A HEPATOMA CELL LINE HTC.

Makowske M, Christensen HN
(c/o Christensen) Dept. Biological Chemistry, Univ. Michigan Medical Sch., Ann Arbor, MI, 48109
J Biol Chem; 257(10):5663-5670 1982

The uptake of anionic amino acids by the rat hepatoma cell line, HTC, occurs by two saturable routes, one Na+-dependent and one Na+-independent. Which of the two mediations will serve for transport of a given anionic amino acid depends on the chain length of that anionic substrate. The Na+-independent system, shared by glutamate, cysteine, and anionic amino acids intermediate in length between the two, is stereoselective, pH-independent, and not inhibited by neutral amino acids. The pH-independent inhibition of this system by LL-beta-mercaptolactate-cysteine disulfide confirms that cystine reacts as an anionic amino acids. Shorter anionic amino acids, including aspartate, are scarcely transported by this route. The accommodation of the cystine molecule calls for its stabilization as an anion even below pH 7. Inhibition triple that of cysteine by the product of its hydrolytic monodeamination confirms this interpretation. A Na+-independent system similar to the one described here has been reported for fetal human lung fibroblasts. In contrast, the Na+-dependent route for anionic amino acid uptake by HTC cells is shared only by the three short anionic amino acids, aspartate, cysteate, and cysteinesulfinate, among the analogs tested. Characteristics of the latter system include an unexplained type of pH dependence and inhibition by threonine and other amino acids known to be substrates for System ASC. Both systems for anionic amino acid uptake in the HTC cell have properties different from those of the two saturable, Na+-dependent routes of anionic amino acid uptake previously described for the cultured rat hepatocyte. (Author abstract) (28 Refs)

75. CONTRIBUTIONS OF SECRETED TUMOR CELL PRODUCTS TO METASTASIS.

Roblin R

Cancer Biology Program, Frederick Cancer Res. Center, Frederick, MD

Cancer Biol Rev; 2:59-94 1981

The roles of products secreted by tumor cells whose action could play a role in the neovascularization, intravasation, capillary arrest, and extravasation stages of hematogenous metastasis are reviewed. Tumor angiogenesis factors, including the properties of epidermal growth factor and fibroblast growth factor, and hydrolytic enzymes, including plasminogen activator, collagenase, lysosomal hydrolase, and procoagulants are discussed. (148 Refs)

76. HUMAN TRANSFERRIN RECEPTOR: EXPRESSION OF THE RECEPTOR IS ASSIGNED TO CHROMOSOME 3.

Enns CA, Suomalainen HA, Gebhardt JE, Schroder J, Sussman HH
(c/o Sussman) Lab. Experimental Oncology, Dept. Pathology, Stanford Univ. Sch. Medicine, Stanford, CA, 94305
Proc Natl Acad Sci USA; 79(10,part1):3241-3245 1982

Human chromosome 3 had been identified as responsible for expression of the transferrin receptor in mouse-human lymphocyte hybrids. The receptor was detected by immunoprecipitation with anti-human receptor antibody of 125I-labeled cells. This method also detected a similar 94,000-dalton protein in mouse cells. A radioimmunoassay developed for the human transferrin receptor measured 10% crossreactivity with the mouse protein. The two proteins were distinguished by NaDodSO4/polyacrylamide gel patterns of partial proteolytic digests of the immunoprecipitated proteins. Mouse-human hybrids were generated by fusing a mouse thymoma (BW5147) cell line to either concanavalin A- or pokeweed mitogen-activated human peripheral blood lymphocytes or a mouse myeloma (NS-1) to uncultured human peripheral blood lymphocytes. Each hybrid was karyotyped with respect to both mouse and human chromosomes. In every case, expression of the human transferrin receptor correlated only with human chromsome 3. (Author abstract) (32 Refs)

77. PUTRESCINE AS A REGULATOR OF EPSILON-(GAMMA-GLUTAMYL)LYSINE ISOPEPTIDE PRODUCTION AND THE PROLIFERATIVE STATE.

Patterson MK, Maxwell MD, Birckbichler PJ, Conway E, Carter HA
Biomedical Div., Samuel Roberts Noble Foundation, Inc., Ardmore, OK, 73401
Cell Biol Int Rep; 6(5):461-470 1982

Inhibition of isopeptide bond formation in WI38 human embryonic lung cells upon putrescine treatment, and stimulation of isopeptide synthesis in WI38 VA13A cells after administration of the putrescine synthesis inhibitor 1,3 diaminopropanol, are reported. The results support a model in which elevated levels of isopeptide are associated with the proliferative state. (52 Refs)

78. DIFFERENCES IN GROWTH REGULATION OF NORMAL AND TUMOR CELLS (MEETING ABSTRACT)

Pardee AB, Campisi J, Croy RG
Sidney Farber Cancer Institute, Boston, MA
Conference on Cell Proliferation, Cancer and Cancer Therapy, February 17-19, 1982, New York, New York New York Academy of Sciences; :A13 1982

Normal cells cannot initiate DNA synthesis yet complete their division cycle under inadequate external conditions. The sensitive biochemical event is proposed to be accumulation of protein which in adequate amounts permits entry into S phase, after about 2 hr, and completion of the cycle. Instability of the protein (half life about 2.5 hr) creates a dynamic state so that accumulation depends on rates of both synthesis and degradation. Neoplastic cells may show poorly regulated growth by synthesizing more of this protein or degrading it less rapidly, under conditions that limit normal cells' growth. Overproduction can result from more copies of structural gene per cell, adjacent promoter of high activity, or autoproduction of growth factors. Less rapid degradation could result from less protease activity, or from stabilizing modifications of the protein. Alterations of protein metabolism by these mechanisms have been described. Thus, diverse transforming agents acting via different mechanisms could affect a common regulatory process leading to neoplasia. (no Refs)

79. DETECTION, IDENTIFICATION, AND PARTIAL CHARACTERIZATION OF SERINE PROTEASES AND ESTERASES IN BIOLOGICAL SYSTEMS.

Scheiner CJ, Quigley JP
(c/o Quigley) Dept. Microbiology and Immunology, State Univ. New York, Downstate Medical Center, Brooklyn, NY, 11203
Anal Biochem; 122(1):58-69 1982

Serine proteases are important catalytic regulators of cellular and extracellular function. Tritium-labeled diisopropylfluorophosphate ([3H]-DFP) has been used to stoichiometrically label serine proteases and the labeled proteases analyzed by autoradiography following sodium dodecyl sulfate-polyacrylamide gel electrophoresis. By preincubating a serine protease, or a complex biological mixture containing serine proteases, with specific, synthetic, active-site inhibitors, the covalent incorporation of [3H]DFP into the protease is prevented or substantially reduced. Since the synthetic inhibitors are amino acid or peptide analogs that reflect the preferred cleavage site of a specific serine protease, any inhibitor-dependent reduction in [3H]DFP incorporation indicates the substrate specificity of the given serine protease. Inhibitor profiles (reflected in polyacrylamide gel autoradiographs) for the different classes of serine proteases have been generated by using a variety of purified serine enzymes preincubated with different inhibitors prior to, and during, [3H]DFP labeling. These profiles were then compared to the more complex gel patterns obtained when cell lysates or conditioned culture medium were labeled with [3H]DFP in the presence or absence of the different active-site inhibitors. A number of known and heretofore undescribed serine proteases were detected in various biological systems including transformed fibroblasts, activated macrophages, and mutant strains of Escherichia coli. This technique allows not only for the detection of serine enzymes in complex mixtures, but also indicates that their mol wt and substrate specificity. This method allows for the quantitative detection of 10(-13) mol of a serine protease. The additional use of this technique to screen for naturally occurring inhibitors of serine proteases is discussed. (Author abstract) (37 Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Abita JP 34
Adamson ED 57
Ahmad J 36
Akogyeram C 73
Alexander A 60
Alhonen-Hongist 71
Amiya K 25
Andrews GK 31
Atmar VJ 72
Attramadal A 33
Austen KF 63
Banka CL 56
Banks WL 69
Barritault D 60
Barsky SH 28
Beavo JA 9
Birckbichler PJ 77
Bollum FJ 6
Borun TW 3
Boullie MC 23
Bourrillon R 43
Bourry J 68
Branum EL 35
Briggs RC 16
Brinkley BR 16
Buldakov LA 64
Burtin P 30
Buxbaum JN 60
Byus CV 7
Byus CV 8
Calarco PG 56
Caldani C 68
Campisi J 78
Carter D 27
Carter HA 77
Chamberlain JW 59
Chan W 69
Charpentier R 3
Cheroutre H 61
Christensen HN 74
Claflin AJ 55
Clemmensen I 22
Coelho R 15
Conklyn MJ 52
Conway E 77
Cox GS 62
Criss WE 40
Criss WE 73
Cristofalo VJ 3
Croce CM 3
Croy RG 78
Davis JL 36
Degrave W 61
Delpech A 23
Delpech B 23
Devos R 61
Dilley WG 38
Doherty PJ 9
Duffy PE 17
Elias L 4
Enns CA 76
Evans DJ 19
Fiers W 61
Fletcher MA 55
Fletcher WH 7
Fletcher WH 8
Fondaneche MC 30
Frangione B 60
Franklin EC 60
Gasiewicz TA 37
Gautvik KM 33
Gauville C 34
Gazitt Y 20
Gebhardt JE 76
Georgiade NG 38
Girard N 23
Goding JW 42
Goldstein S 59
Gustafsson JA 41
Hanaoka H 11
Hannan GN 58
Harley CB 59
Hartmann KU 46
Haug E 33
Haustein D 46
Hayashi H 29
Hendrickson MF 12
Hinkle PM 39
Hirashima M 29
Hirostu Y 29
Hnilica LS 16
Holtta E 71
Holund B 22
Hood L 60
Horiuchi Y 11
Hormann H 51
Hoyt BJ 19
Huang YY 17
Huber C 45
Hunter JB 36
Huppe T 46
Ichikawa Y 13
Ikuta T 21
Iri H 11
Ishibashi H 21
Istin M 15
Jackson AG 36
Janne J 71
Jannotta F 28
Jefferson LS 5
Jones G 36
Junker P 22
Kakiuchi S 40
Kalbag RM 47
Kalistratova VS 65
Kalmykova ZI 64
Kanai Y 1
Kaplan AM 69
Katada T 50
Katki AG 12
Katsunuma T 24
Kennedy DW 26
Killingsworth L 32
Kimura JH 63
Kinsella PA 39
Kissonerghis AM 18
Klein G 20
Kobata A 44
Kovi J 36
Kozlovskis PL 55
Krim M 72
Kudo J 21
Kuehn GD 72
Kurl RN 41
Lakshmi MS 48
Latner AL 48
Lauret P 23
Leiter E 45
Liederman L 73
Lin YL 6
Liotta LA 28
Lipson LG 10
Lohmander LS 63
Longmire J 4
Lorscheider FL 31
Lund J 41
Lutz D 45
Lyon H 22
MacLeod MC 2
Madri J 27
Maeda S 44
Mahler PA 67
Makowske M 74
Mansfield BK 2
Maruya M 11
Matsumoto A 44
Maxwell MD 77
McAuslan BR 58
McCarty KS 38
McCarty KS 38
McElhanon S 69
McKinney EC 55
Michmayr G 45
Milano G 68
Miller EE 70
Mitomi T 24
Mizoguchi A 44
Mohla S 36
Moldenhauer G 46
Mori M 11
Morinaga T 31
Morishita Y 73
Moses HL 35
Mullen JL 70
Mumby MC 9
Munsch N 30
Myers CE 12
Naess O 33
Nagata K 13
Nakada Y 25
Nakasaki H 24
Nato F 43
Nelson WJ 14
Nifatov AP 64
Ogoshi K 24
Oka T 73
Okubo H 21
Oldham SB 10

Ono K 25
Owen MJ 18
Palade GE 53
Panrucker DE 31
Pardee AB 78
Pardue RL 16
Park KH 62
Patterson MK 77
Pegg AE 5
Poellinger L 41
Pollard JW 59
Potter CL 66
Potter CW 49
Presti M 70
Pritchard ML 5
Quigley JP 79
Rapport MM 17
Ratliff R 4
Raux H 30
Rees RC 49
Richter H 51
Robinson RA 35
Roblin R 75
Rubin RW 55
Rucci G 37
Russell DH 66
Saal F 34
Sagara J 13
Sahai A 73
Sakihara H 11
Sarris AH 53
Sasaki R 6
Scheiner CJ 79
Schimmer BP 9
Schmidt WN 16
Schneider M 68
Schroder J 76
Schwartz LB 63
Sekar V 72
Selkirk JK 2
Seppanen P 71
Sherbet GV 47
Sherbet GV 48
Shibata K 21
Shimizu N 25
Shiraishi N 44
Siegal GP 28
Silber R 52
Silva JS 38
Sipes IG 66
Stanners CP 59
Stein TP 70
Steinberg JJ 70
Steinmetz M 60
Stevens RL 63
Strauchen JA 54
Sugiyama T 44
Sulitzeanu D 20
Suomalainen HA 76
Sussman HH 76
Tajima T 24
Takaku F 6
Takaoka K 25
Tamaoki T 31
Tan KB 3
Tanuma S 1
Tarin D 19
Taya Y 61
Tietjen GE 10
Tokarskaia ZB 64
Traub P 14
Tsao J 9
Tsuda M 24
Tsuda-Kawamura 21
Tutt MC 16
Ui M 50
Van Heuverwyn H 61
Vedeneev VS 64
Volkenant ME 35
Wagner A 46
Walker JR 49
Wells SA 38
Wildridge M 47
Wood A 4
Yatvin MB 67
Ydenberg RC 19
Yoshikawa H 25
Yoshima H 44

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In this issue:

- NUCLEOPROTEINS........ 1-8
- CYTOPLASMIC AND CONTRACTILE PROTEINS........ 9-19
- PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS........ 20-38
- BINDING/ RECEPTOR PROTEINS........ 39-47
- MEMBRANE/ SURFACE PROTEINS........ 48-56
- STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS........ 57-62
- EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM........ 63-73
- DIETARY AND NUTRITIONAL EFFECTS........ 74
- POLYAMINES - SYNTHESIS AND METABOLISM........ 75-77
- OTHER RELATED STUDIES........ 78-83

NUCLEOPROTEINS

1. THE CHARACTERIZATION OF NON-HISTONE PROTEINS WHOSE AMOUNTS INCREASE IN CHROMATIN FROM MOUSE HEPATOCARCINOMAS.

Medvedev ZA, Buchanan JH, Medvedeva MN, Crowne HM

Div Genetics, Natl. Inst. Medical Res., Mill Hill, London NW7 1AA, England

Int J Cancer; 30(1):87-92 1982

A fraction containing liver- and hepatoma-specific non-histone proteins has been isolated from the chromatin of mice. Amino acid analysis of this fraction shows that it contains 16 mol of glutamic acid, 10 mol aspartic acid, 7 mol of both arginine and lysine per 100 mol and contains no cysteine or tyrosine. The proteins in this fraction are strongly associated with DNA and are co-extracted with histones from chromatin with 0.25 M HCl. In chromatin from age-related hepatomas, the amount of this fraction increased six-fold. This increase in concentration of these chromatin proteins may be associated with changes of chromatin structure necessary to initiate malignant growth in liver cells. (Author abstract) (33 Refs)

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2. PROTEINS AND FUNCTIONAL PROPERTIES OF CHROMATIN FRACTIONS FROM NUCLEI OF NORMAL LIVER AND EXPERIMENTAL HEPATOMAS.

Lobanenkov VV, Mironov NM, Shapot VS
Lab. Tumor Biochemistry, All-Union Cancer Res. Center, Moscow, USSR
Biull Eksp Biol Med; 93(5):60-63 1982

Functional activity of chromatin fractions was studied in Wistar rats bearing solid hepatoma 27 or ascites Zajdela hepatoma and in C3HA mice bearing solid hepatoma 22 or ascites hepatoma 22a. The nuclei from both normal liver and hepatomas contained chromatin fragments with endogenous RNA-synthesizing activity. The chromatin fragments were associated with nuclear matrix and were enriched with nonhistone proteins. (13 Refs) (In RUS)

3. ALKYLATION OF NUCLEAR PROTEINS IN RAT LIVER BY DIETHYLNITROSAMINE.

Letnansky K
Inst. Cancer Res., Univ. Vienna, Borschkegasse 8a, A-1090 Vienna, Austria
Cell Mol Biol; 28(3):353-361 1982

After the injection of N,N-di[1-14C]-ethylnitrosamine into hepatectomized rats incorporation of radioactive label into histone and non-histone proteins of liver nuclei is demonstrated. Differences are observed, however, between the alkylation patterns of nuclear proteins from chromatin enriched in transcriptionally active sequences as compared to inactive chromatin. Although many protein components are alkylated in both fractions, the increased incorporation of ethyl residues into histone H2B and the diminished alkylation of histone H1' of the active sequences are particularly striking. Similarly, H2B is ethylated only in the mononucleosomal fraction of nuclease digests which consists of particles released from actively transcribed sequences. While differences are less pronounced between H1' histones from monomeric and oligomeric particles, clear differences were demonstrated with respect to the incorporation of ethyl residues into certain non-histone proteins. (Author abstract) (58 Refs)

4. DNA-BINDING PROTEIN FROM HELA CELLS THAT BINDS PREFERENTIALLY TO SUPERCOILED DNA DAMAGED BY ULTRAVIOLET LIGHT OR N-ACETOXY-N-ACETYL-2-AMINOFLUORENE.

Tsang SS, Kuhnlein U
(c/o Kuhnlein) Environmental Carcinogenesis Unit, British Columbia Cancer Res. Center, 601 West 10th Ave., Vancouver, BC V57 1L3, Canada
Biochim Biophys Acta; 697(2):202-212 1982

A DNA-binding protein was partially purified from extracts of HeLa cells by high-speed centrifugation and chromatography on DEAE-cellulose, phosphocellulose and ultraviolet light-irradiated DNA-cellulose columns. It eluted from the phosphocellulose column with 0.375 M potassium phosphate and from the ultraviolet light-irradiated DNA-cellulose column between 0.5 M and 1 M NaCl. The protein binds preferentially to supercoiled PM2 DNA treated with ultraviolet light or N-acetoxy-N-acetyl-2-aminofluorine, as compared to native supercoiled PM2 DNA. The binding is non-cooperative. Nicked or linear forms of PM2 DNA (damaged or untreated) are not efficient substrates, indicating a requirement of DNA supercoiling for DNA binding. The sedimentation coefficient of the protein estimated by glycerol gradient centrifugation is 2.0-2.5 S, corresponding to a molecular weight of about 20,000-25,000 if the protein is spherical. The binding to DNA irradiated with ultraviolet light or treated with acetoxyacetylaminofluorene is optimal at around 100-200 mM NaCl and is relatively independent of temperature and pH. MgCl2 and MnCl2 at concentrations between 1 and 5 mM do not markedly affect the binding, but it is inhibited by sucrose, ATP and caffeine. The biological significance of the DNA-binding protein remains to be determined. It does not possess significant glycosylase, endonuclease or exonuclease activities. The dissociation equilibrium constant for the binding reaction of the protein to the ultraviolet light or acetoxyacetylaminofluorene-induced binding sites on DNA is estimated to be 4 x 10(-11) M. There are at least 1 x 10(5) DNA-binding protein molecules/HeLa cell. (Author abstract) (32 Refs)

5. DIFFERENTIAL INHIBITION OF TRANSCRIPTION OF DNA BY MELANOMA CHROMOSOMAL PROTEINS.

Lambert MW, McGuire J
Dept. Pathology, UMDNJ-New Jersey Medical Sch., 100 Bergen St., Newark, NJ, 07103
J Invest Dermatol; 78(6):498-502 1982

Histones and four nuclear nonhistone protein fractions (NHP1-4) were extracted from nuclei of a Cloudman mouse melanoma cell line (NCTC 3960, CCL 53) and tested for their ability to bind to DNA and influence transcription. The histones and NHP fractions showed different binding affinities for DNA, with the histones and NHP1 exhibiting the highest affinity. The NHP fractions differentially affected both the rate of RNA synthesis and the size of RNA transcribed. NHP1 which inhibited RNA synthesis to the greatest extent, inhibited synthesis of all sizes of RNA except for major peaks of 28S and 8S RNA and discrete minor peaks of 7S, 6S, 5S, and 4S RNA. Histones markedly enhanced the effect of NHP1 on RNA synthesis. These results suggest that there are nonhistone proteins in Cloudman melanoma nuclei which have a high affinity for DNA and which may be involved in the regulation of transcription. (Author abstract) (37 Refs)

6. IMMUNOLOGICAL SPECIFICITY OF NOVIKOFF HEPATOMA CHROMATIN: ISOLATION OF THREE ANTIGENIC PROTEINS.

Stryjecka-Zimmer M, Schmidt WN, Briggs RC, Hnilica LS
Dept. Physiological Chemistry, Medical Sch., Lubartowska 85, 20-123 Lublin, Poland
Int J Biochem; 14(7):591-600 1982

Rabbits immunized with dehistonized Novikoff hepatoma (NH) or normal rat liver (NRL) elicited specific antibodies. Fractionation of nonhistone proteins from the NH group yielded three proteins (approx mol wt 39,000, 49,000 and 56,000) identified with the hepatoma antigenic complexes which showed significant reactivity with PAS staining, suggesting they were glycosylated. Three similar proteins from NRL were PAS negative, and did not react immunologically with antiserum to dehistonized NH chromatin. (49 Refs)

7. ELECTROPHORETIC STUDIES OF DNASE IN PROLIFERATIVE AND TUMOROUS TISSUES.

Sakata T
Dept. Medical Biochemistry, Kurume Univ. Sch. Medicine, Kurume, Fukuoka Pref. 830, Japan
Kurume Igakkai Zasshi; 44(12):858-868 1981

Under normal keratinization of epidermis, the cellular nuclei disappear from the border of the stratum corneum, where no DNA is detectable. Epidermal DNase is known to play a role in degradation of the nucleic acid. To find the physiological significance of DNase, the normal epidermis, hexadecane-induced hyperkeratotic epidermis and dimethylbenzanthracene-induced papilloma of mice were studied. Electrophoretic properties of DNase were analyzed by the methods of micro disc electrophoresis and U-tube isoelectric focusing. (1) Concerning the enzyme activity, acid DNase was two times higher than neutral DNase in the normal epidermis. However, there was a lower activity of acid DNase in the hyperkeratotic epidermis and papilloma. A remarkable decrease in neutral DNase was found in the papillomatous tissue. (2) The different patterns in electrophoresis were not observed among the abnormal epidermis, hyperkeratotic epidermis and papilloma. (3) Isoelectric point was pH 7.2 in acid DNase and pH 4.7 in neutral DNase. Any significant difference of isoelectric points was not observed in the normal and abnormal epidermis. From these results, it was suggested that there was a decrease in DNase activity in the proliferative and tumorous tissues as compared to normal but no differences were detected by the method of electrophoresis. (Author abstract) (32 Refs) (In JPN)

8. STIMULATION OF HISTONE PHOSPHORYLATION BY 12-O-TETRADECANOYL-PHORBOL-13-ACETATE (TPA) IN PRIMARY MOUSE SPLEEN CELL CULTURES (MEETING ABSTRACT).

Patakan GJ, Baxter CS

Department of Environmental Health, University of Cincinnati, 3223 Eden Avenue, Cincinnati, OH, 45267

Proc Am Assoc Cancer Res; 23:40 1982

Certain phorbol diesters are potent tumor-promoting agents and mitogens in mouse epidermis, and stimulate histone phosphorylation in this tissue. This response may thus be a reflection of cell proliferation or be specifically involved in the mechanism of promotion. Phorbol diester effects on histone phosphorylation in a system responsive in other ways but not to the mitogenic activities of these agents, were therefore examined. Murine splenocyte suspensions were pulsed with 32P-inorganic phosphate for 2 hr, nuclei isolated and the acid-extracted histones fractionated by one- and two-dimensional polyacrylamide gel electrophoresis. TPA treatment of the splenocytes resulted in dose-dependent increases in the phosphorylation of histones H1, H2A, H3 and H2B specifically, over the range of 17 to 1700 nM. Two hr after a promoter treatment of 1700 nM increases relative to controls were: H1 (220%), H2A (140%) and H3 (169%). Phosphorylation of H2B and H4 was not observed in control cells, whereas H2B phosphorylation was induced only in promoter treated cells. The stimulation observed for all histones was inhibited by retinoic acid (25 uM). The promoter-induced phosphorylation of histone H2B was furthermore not observed after cell exposure to the mitogen phytohemagglutinin. These results suggest that a tumor-promoting agent can induce specific changes in histone phosphorylation in the absence of cell proliferation, and that the system described may be valuable for determining the relevance of this biochemical response in tumor promotion. (no Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

9. BIOCHEMICAL AND IMMUNOCYTOCHEMICAL ANALYSIS OF THE INTERMEDIATE FILAMENT CYTOSKELETON IN HUMAN HEPATOCELLULAR CARCINOMAS AND IN HEPATIC NEOPLASTIC NODULES OF MICE.

Denk H, Krepler R, Lackinger E, Artlieb U, Franke WW

Div. Gastroenterologic and Molecular Pathology, Univ. Vienna, Spitalgasse 4, A-1090 Wien, Austria

Lab Invest; 46(6):584-596 1982

Cytoskeletons enriched in intermediate-sized filaments were isolated from neoplastic and non-neoplastic hepatocytes of murine and human liver as well as from human cholangiocellular carcinoma, using extractions in high salt buffers and Triton X-100, and were analyzed by immunofluorescence microscopy and by one- and two-dimensional gel electrophoresis. This study presents the first comparison of the cytoskeletal pattern of a human nonepidermal tissue and the corresponding carcinoma. Despite some minor but significant differences of electrophoretic mobilities of the major cytokeratin polypeptides present in rodent and human liver, their similar pattern on two-dimensional gel electrophoresis suggests a tissue or cell type-specific pattern in both species. Moreover, the results show that not only cytokeratins are continued to be expressed when hepatocytes become neoplastic but also that the hepatocyte-specific pattern of major cytokeratin polypeptides is maintained. (Author abstract) (45 Refs)

10. LOCALIZATION OF S-100 PROTEIN AND GLIAL FIBRILLARY ACIDIC PROTEIN-RELATED ANTIGEN IN PLEOMORPHIC ADENOMA OF THE SALIVARY GLANDS.

Nakazato Y, Ishizeki J, Takahashi K, Yamaguchi H, Kamei T, Mori T

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Lab Invest; 46(6):621-626 1982

Pleomorphic adenoma of the salivary glands was studied by the peroxidase-antiperoxidase method and Ouchterlony's double diffusion for the presence of so-called nervous system-specific proteins, S-100 protein, glial fibrillary acidic protein (GFAP), and astroprotein. Positive immunostaining for S-100 protein was observed in tumor cells of epithelial, myxomatous, and chondroid areas. An immunodiffusion test confirmed its presence in the tumor. Normal salivary glands were also stained with anti-S-100 serum, but the reaction was less intense than that of tumor. Specific immunostaining for GFSAP and astroprotein was found in a small number of cells of myxomatous and chondroid areas and occasionally in cells in epithelial areas. An immunodiffusion test suggests that the GFAP-related antigen of the pleomorphic adenoma showing a minor heterogeneity of antigenic determinants is almost identical with GFAP. Normal salivary glands did not stain with GFAP or astroprotein antisera, nor did they react with anti-GFAP serum by immunodiffusion. Thus, the S-100 protein and GFAP-related antigen may be actively synthesized in adenoma cells during the course of tumor development. In addition, the GFAP-related antigen is considered to be a tumor-associated antigen of pleomorphic adenoma of the salivary glands. (Author abstract) (28 Refs)

11. A NEW PROTEIN FACTOR INHIBITING ACTIN-POLYMERIZATION IN LEUKEMIC MYELOBLASTS.

Nagata K, Sagara J, Ichikawa Y
Dept. Cytochemistry, Chest Disease Res. Inst., Kyoto Univ., Kyoto 606, Japan
Cell Struct Funct; 7(1):1-7 1982

Polymerization of actin was inhibited in the cytoplasm of D-line cells, which had lost their ability to differentiate to motile cells, and which were derived from a myeloid leukemia cell line (M1). The actin polymerization inhibitor (API) isolated from the D- cells was trypsin sensitive, heat labile and had a molecular wt of about 71,000. The API did not depolymerize F actin. (21 Refs)

12. PURIFICATION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE OF 60,000 DALTON FROM MAMMALIAN THYMUS AND THYMOMA BY IMMUNOADSORBENT COLUMN AND COMPARISON OF PEPTIDE STRUCTURES.

Nakamura H, Tanabe K, Yoshida S, Matsuyama M, Morita T
Dept. Experimental Radiology, Aichi Cancer Center Res. Inst., Chikusa-ku, Nagoya 464, Japan
Adv Exp Med Biol; 145:19-23 1982

Immunoadsorbent column chromatography, which is suitable to purify terminal deoxynucleotidyl transferase (TdT), was developed to study the differences in the mol wt of TdT and investigate whether the differences reflect species- or tissue-specificities of the enzyme, or are due simply to the proteolytic degradation during purification process. Results suggested that a single polypeptide with 60,000 dalton (60-k) must be the original form of mammalian TdT, and the alpha and beta subunits of the enzyme might be proteolytic products derived from the 60-k form. Tryptic peptide maps suggested homology in amino acid sequences among the TdT's from three mammalian species. (10 Refs)

13. GROWTH RATE-LINKED DECREASE IN PYRUVATE DEHYDROGENASE COMPLEX (PDC) ACTIVITY IN HEPATOMAS (MEETING ABSTRACT).

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Laboratory for Experimental Oncology, Indiana University School of Medicine, Indianapolis, IN, 46223
Proc Am Assoc Cancer Res; 23:59 1982

The purpose of this investigation was to test whether the reprogramming of gene expression in neoplasia required a decrease in the activity of PDC, which has a key role in channeling pyruvate into the tricarboxylic acid cycle. Homogenates were prepared in potassium phosphate buffer, pH 7.4, containing glycerol and PDC activity was assayed under linear kinetic conditions by measuring the evolution of 14CO2 from [1-14C] pyruvate. In normal liver the activity of the PDC was 1.25 umol/hr/mg protein. In slowly growing rat hepatomas 20 and 16, in the medium growth rate tumors 7787, 9618AM, 44 and 8999 and in rapidly growing hepatomas 5123D, 7800, 5123 tc, 3924A and 7777 the PDC specific activity was significantly decreased to 76, 89, 91, 68, 36, 47, 56, 27, 62, 38 and 27%, respectively, of values of livers of normal rats of the same sex, age and weight as the tumor bearing rats. The results were consistent with the report that in the rapidly growing hepatoma 3924A the acetyl CoA concentration was decreased to 21% of that of normal liver. PDC specific activity in the differentiating liver of 6-day-old rats was 35% of that of the normal, adult rat. In 24 and 48 hr regenerating liver specific activity was 62 and 86%, respectively, of that of sham-operated ones. The demonstration of decreased PDC activity in all hepatomas examined, in parallel with the growth rate, suggests that the reprogramming of gene expression in malignant progression is linked with a decrease in the activity of this enzyme. (1 Ref)

14. PURIFICATION AND CHARACTERIZATION OF GALACTOSYLTRANSFERASE (GT) FROM ASCITES OF OVARIAN CANCER PATIENTS (MEETING ABSTRACT).

Chatterjee SK, Bhattacharya M, Barlow JJ
Roswell Park Memorial Institute, Buffalo, NY, 14263
Proc Am Assoc Cancer Res; 23:73 1982

GT appears to be an excellent marker for ovarian cancer. With the aim of developing a radioimmune assay for GT, the enzyme was purified from ascites of ovarian cancer patients using the following steps: 1) 20-50% ammonium sulfate fractionation, 2) affinity chromatography in lactalbumin-Sepharose 4B column, 3) affinity chromatography in N-acetyl-glucosamine-Sepharose 4B column and 4) affinity chromatography in a second lactalbumin-Sepharose 4B column. A final purification of over 100,000 fold with an overall yield of about 20% was achieved. During purification, the enzyme rapidly lost activity if Triton X-100 was omitted from the media. Dilution of Triton X-100 lower than 0.01 in the medium of purified or semi-purified enzyme preparation caused inactivation of the enzyme activity. Purified GT showed a single thick protein band in sodium dodecyl sulfate-gel electrophoresis with an average Mr of 51,000. Gel electrophoresis in alkaline conditions showed one diffused band, and a small sharp slower moving band. A single protein band was obtained by gel electrophoresis in acidic conditions. When purified GT was chromatographed on a column of Concanavalin A-Sepharose 4B, a fraction of the enzyme activity remained bound to the column and could be eluted with alpha-methyl-mannoside, suggesting the presence of multiple components in the purified preparation. (no Refs)

15. A RADIOIMMUNOASSAY FOR DIHYDROFOLATE REDUCTASE AND ITS APPLICATIONS TO BIOLOGICAL SYSTEMS.

Iqbal MP
New York University, New York, NY
Diss Abstr Int [B]; 42(12,pt1):4652-B 1982

Dihydrofolate reductase (DHFR) was purified to homogeneity from calf liver and L1210 murine leukemia cells by affinity chromatography. A sensitive and specific radioimmunoassay for DHFR was developed using 125I-labeled calf liver DHFR and rabbit and chicken antisera to the purified calf and L1210 DHFR, respectively. A functionally inactive but immunoreactive form of DHFR has been identified in human leukemia cells and normal leukocytes, in human normal and cancerous tissues and in L1210 murine leukemia cells using this radioimmunoassay. The concentration of this immunoreactive nonfunctional protein in all of these cells was substantially greater than the concentration of active DHFR as measured by the stoichiometric binding of [3H]-methotrexate. By gel filtration, the nonfunctional immunoreactive form of DHFR in both human chronic myelogenous leukemia cells and L1210 leukemia cells has an apparent molecular weight of 41,000 and 318,000, respectively. The active enzyme from both cell types has a molecular weight of approximately 20,000. Isoelectrofocusing of cytosol from L1210 cells separated two immunoreactive isoproteins, each corresponding exactly to the same isoelectric points of the two active isoenzymes suggesting that this high molecular weight form is comprised of subunits of active enzyme. The nonfunctional immunoreactive protein in L1210 leukemia cells has a reciprocal relationship with active DHFR during the in vivo log phase of growth, suggesting that it may be a precursor form of the active enzyme. (no Refs)

16. MULTIPLE FORMS OF AROMATASE AND RESPONSE OF BREAST CANCER AROMATASE TO ANTIPLACENTAL AROMATASE II ANTIBODIES.

Osawa Y, Tochigi B, Higashiyama T, Yarborough C, Nakamura T, Yamamoto T
Endocrine Biochemistry Dept., Medical Foundation Buffalo, Inc., Buffalo, NY, 14203
Cancer Res; 42(8,Suppl):3299s-3306s 1982

Two distinct aromatase-active protein complexes are solubilized by use of deoxycholate and separated by diethylaminoethyl-cellulose chromatography from lyophilized powder of 900 x g precipitate fraction of human term placenta. Aromatase activity to produce estriol, the major estrogen of human pregnancy, was designated to be aromatase I activity and measured by estriol formation from 1-6alpha-hydroxytestosterone. Aromatase II activity was the designation for that which produces estrone plus estradiol and was measured by androstenedione aromatization. Aromatases II and I are eluted with 0.25 M and 0.5 M Tris buffer, respectively, from diethylaminoethyl-cellulose column in an Mr 2 million soluble complex. Each has a minimum active Mr 135,000 subunit, which is isolated by Bio-Gel filtration in the presence of detergents, and consists of a reduced nicotinamide adenine dinucleotide phosphate:cytochrome P-450 reductase (Mr 83,000) and a cytochrome P-450 (Mr 52,000). Aromatase II was found to be the major aromatase, containing approximately 5x more aromatase activity, reduced nicotinamide adenine dinucleotide phosphate:cytochrome c reductase activity, cytochrome P-450, and protein than did aromatase I. Antibodies raised in rabbits against aromatase II and its reductase suppressed aromatase II activity of breast cancer tissues, as well as of adult male lung tissue, placental microsomes, and solubilized aromatase. The breast carcinoma specimens responded to the antibodies in different degrees, but there was no response to antibodies against rat liver cytochrome P-450. The results indicate similar antigenic structures for breast cancer and placental aromatase but not for rat liver cytochrome P-450. (Author abstract) (29 Refs)

17. IMMUNOHISTOCHEMICAL LOCALIZATION OF HUMAN LIVER ALCOHOL DEHYDROGENASE IN LIVER TISSUE, CULTURED FIBROBLASTS, AND HELA CELLS.

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Medizinisch Chemisches Institut der Universitat, Postfach, CH-3000 Bern 9, Switzerland
Am J Pathol; 108(1):89-99 1982

Human liver alcohol dehydrogenase (ADH, EC 1.1.1.1) was purified by double ternary complex affinity chromatography on Sepharose-4(3-[N-6 aminocaproyl]aminopropyl) pyrazole. The purified enzyme preparation still contains several isoenzymes reflecting the isoenzyme composition of the starting material. Antibodies against this mixture of isoenzymes were elicited in rabbits. The specificity of the antiserum was tested by double immunodiffusion, enzyme-linked immunosorbent assay, immunoprecipitation of ADH enzymatic activity, and adsorption to ADH, which was immobolized to Ultrogel AcA 44 by the use of glutardialdehyde as the coupling agent. Protein-A peroxidase with diaminobenzidine or amino ethyl carbazole as substrate, served to detect binding of anti-human liver ADH antibodies in human liver thin sections, cultured human skin and lung fibroblasts, and HeLa cells. Fluorescein-conjugated antibodies were also used in direct immunofluorescence on liver tissue. In the human liver, ADH was found to be localized in the cytoplasm of hepatocytes. Differences in the staining intensity of hepatocytes may reflect differences in ADH content. Strongly stained hepatocytes were localized mainly around the central veins. Perinuclear staining is often seen, especially in the more lightly stained cells. Human skin and lung fibroblasts, as well as HeLa cells, all exhibited positive staining for ADH. The pattern was identical to that found in hepatocytes, although the staining intensity was much weaker, indicating a lower ADH content. (Author abstract) (41 Refs)

18. CYTOKERATINS OF HUMAN SKIN CARCINOMAS (MEETING ABSTRACT).

Reano A, Viac J, Thivolet J
Laboratoire de Recherche Dermatologique et Immunologie, Hopital E Herriot-Pav R, INSERM U209, 69374 Lyon Cedex 2, France
J Invest Dermatol; 78(4):331 1982

The cytokeratins of epithelial cells from basal cell carcinomas (8 cases) and squamous cell carcinomas (3 cases) free of surrounding normal tissue, were investigated for their biochemical and immunological properties. Cytokeratin proteins were extracted with high salt buffer and triton x 100 according to a technique described by Winter et al and were comparatively analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Results showed in both tumors either the absence or a very low amount (5% of the total protein) of the major protein band of molecular weight (MW) 67 Kd present in normal human epidermis and confirmed immunolabeling results showing a staining limited to some dyskeratotic cells in cryostat sections of these tumors with an anti 67 Kd serum. In contrast gel electrophoresis showed in both tumors 3 distinct groups of predominant polypeptide bands of apparent relative MW values: (1) 60-65 Kd, (2) 52-55 Kd, and (3) 49 Kd representing respectively about 43%, 31% and 20% of the total proteins. Antibodies raised in animals against polypeptides 49 Kd and 52-55 Kd in a case of a large squamous cell carcinoma, strongly labelled in indirect immunofluorescence malignant cells present in these tumors. On normal human skin, the labelling was preferentially located to the cell cytoplasm of the basal and spinous layers of epidermis whereas anti 67 Kd serum labelled only the upper layers. These results confirmed the heterogeneity of cytokeratins expressed by human keratinocytes during the course of differentiation in epidermis. Although no 'specific' keratin polypeptide seemed to be expressed in skin carcinomas, some major differences existed such as the relative increase of polypeptides of low MW and the very low amount of 67 Kd polypeptide. Such a result is in good correlation with the fact that cells with a mitotic capacity belong to the germinal layer. (no Refs)

19. MOLECULAR EVENTS IN CAMP-MEDIATED REVERSE TRANSFORMATION.

Lockwood AH, Trivette DD, Pendergast M
Dept. Anatomy, Univ. North Carolina Sch. Medicine, Chapel Hill, NC, 27514
Cold Spring Harbor Symp Quant Biol; 46(part2):909-919 1981

Studies of mouse BALB/c 3T3 and Chinese hamster ovary cells demonstrated that phosphorylation and dephosphorylation of specific cytoskeletal and cytoplasmic proteins occur during reverse transformation of transformed cells by cyclic (c)AMP. The phosphoproteins are thought to mediate the acquisition of normal morphology and the organization of the cytoskeleton induced in transformed cells by cAMP. (40 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

20. IMMUNOLOGICAL SIMILARITY OF CEA AND ALPHA1-ACID GLYCOPROTEIN (OROSOMUCOID).

Ochi Y, Fujiyama Y, Hosoda S, Miyazaki T, Yoshimura M, Hachiya T, Kajita Y
Second Dept. Internal Medicine, Shiga Univ. Medical Science, Seta, Otsu, Shiga, 520-21, Japan
Clin Chim Acta; 122(2):145-160 1982

Carcinoembryonic acid (CEA) labeled with 125I could be precipitated by antibody to alpha1-acid glycoprotein (AG) in a dose-dependent manner. CEA preparations showed cross reactivity with three kinds of commercial anti-AG preparations by polyethylene glycol and by a double antibody method. The radioactive peak of 125I-CEA showed cross-reactivity with commercial anti-AG and with antisera to purified AG preparations. The binding of 125I-CEA with anti-AG was displaceable by purified AG. Thus, CEA and AG are immunologically similar, although antibody against CEA showed no cross-reactivity with AG. The CEA molecule may have a component that is immunologically similar to AG and it may be in the protein part. CEA purified from hepatic metastases of a primary adenocarcinoma of the colon was subjected to anti-AG sepharose affinity chromatography, eluted and labeled with 125I. Fractionation revealed a peak with extremely high immuno-reactivity with both anti-AG and anti-CEA and a large molecular protein with both AG and CEA antigenic determinants. Studies of the immunoreactivity of this large mol wt fraction demonstrated that the immunoreaction of CEA with anti-AG was a non-precipitation reaction. Based on differences in binding affinities between CEA with anti-CEA and with anti-AG, it was postulated that CEA may be a biosynthetic precursor of AG or pro-AG. (38 Refs)

21. IMMUNOCHEMICAL DEMONSTRATION OF AN OVARIAN CANCER-ASSOCIATED URINARY PEPTIDE.

Stenman UH, Huhtala ML, Koistinen R, Seppala M
I, II Dept. Obstetrics and Gynecology, Helsinki Univ. Central Hosp., SF-00290 Helsinki 29, Finland
Int J Cancer; 30(1):53-57 1982

Immunization with a peptide fraction from ovarian cancer urine resulted in an antiserum that reacts with a 6,000-dalton (6K) peptide. In urine from some patients with advanced gynecological cancer, the concentration of 6K peptide was high enough to be detected by immunodiffusion. By radioimmunoassay the levels in normal serum could be determined. The concentration in serum was 5-20 ug/l and that in urine 5-50 ug/l. Elevated levels were observed in urine from 5 out of 8 patients with ovarian cancer, 10 out of 14 patients with cervical cancer and 9 out of 14 patients with endometrial cancer. The highest level observed was 12,000 ug/l. High concentrations of the 6K peptide (100-300 ug/l) were observed in amniotic fluid from 14-16 weeks of pregnancy. Some tumor extracts also had a high concentration of the 6K peptide. No immunological relationship was observed between the peptide and known tumor-associated antigens or serum proteins. These results suggest that the peptide is a new oncodevelopmental peptide which may have significance as a tumor marker. (Author abstract) (17 Refs)

22. CALCITONIN IMMUNOREACTIVE PROTEIN, A TUMOR MARKER IN SMALL-CELL BRONCHOGENIC CARCINOMA.

Luster W, Gropp C, Havemann K
Abt. fur Hamatologie und Onkologie, Med. Klinik, Marburg, W.Germany
Verh Dtsch Ges Inn Med; (87):1089-1092 1981

Calcitonin was measured in the serum of 152 patients with lung tumors of various histological types. Protein molecules showing calcitonin activity were characterized by column chromatography. Small-cell bronchogenic carcinoma produced such molecules in proportion to the synthetic capacity of the cells. These molecules probably represent precursors of calcitonin and are suited to use as markers for monitoring and guiding therapy of small-cell carcinoma. (8 Refs) (In GER)

23. ANTIGENS OF THE BASEMENT MEMBRANE AND THE PERITUMORAL STROMA IN HUMAN COLONIC ADENOCARCINOMAS: AN IMMUNOFLUORESCENCE STUDY.

Burtin P, Chavanel G, Foidart JM, Martin E
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Int J Cancer; 30(1):13-20 1982

Twenty colonic adenocarcinomas were studied by immunofluorescence with antisera against components of the basement membrane: Type IV collagen, laminin and heparan sulfate-rich proteoglycan, as well as antisera against antigens of the connective tissue: Type-III collagen, fibronectin and hyaluronectin. Marked alterations of the basement membranes were consistently observed on staining with each one of the first three antisera. In contrast, staining of the normal components of connective tissue was in most cases as intense as in normal colonic mucosa. Hyaluronectin, a marker of peritumoral stroma, was found to be present in 12 out of 15 tumors studied. (Author abstract) (22 Refs)

24. INTERFERON ENHANCEMENT OF THE INVASIVE CAPACITY OF EWING SARCOMA CELLS IN VITRO.

Siegal GP, Thorgeirsson UP, Russo RG, Wallace DM, Liotta LA, Berger SL
(c/o Berger) Lab. Pathophysiology, NCI, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(13):4064-4068 1982

The ability of interferons to reduce cell proliferation in vitro and in vivo is a well-studied phenomenon. To extend such observations, the effect of interferons on the invasiveness in vitro of human malignant cells derived from a Ewing sarcoma was evaluated. Two related parameters were examined: (i) production of Type IV (basement membrane) collagenase and (ii) penetration of human amnion basement membrane and collagenous stroma. After 6 days of treatment with crude fibroblast interferon, leukocyte interferon, or lymphoblastoid interferon at 100 units/ml in serum-free medium, Type IV collagenase levels increased two- to four-fold per cell relative to those of untreated controls. With homogeneous fibroblast and lymphoblastoid interferons, a two-fold elevation in Type IV collagenase was detected after 2 days, with further increases, occasionally dramatic, occurring on the 4th and 6th day of treatment. The ability of Ewing sarcoma cells to invade human amnion connective tissue was measured after 6 days of treatment with various interferons. Relative to the behavior of untreated controls, crude leukocyte interferon, homogeneous lymphoblastoid interferon, and homogeneous fibroblast interferon at 100 units/ml augmented invasiveness 3-, 17-and 22-fold, respectively, when cells were allowed 4 days in which to traverse the amnion. When untreated cells were exposed simultaneously to the amnion and to homogeneous lymphoblastoid or fibroblast interferon, a four- to five-fold increase in invasiveness above control levels was observed in 2 days. These data emphasize the complexity of interferon-induced phenomena. In any overview, the effects of interferon on both the tumor cell and the host must be considered. (Author abstract) (27 Refs)

25. A PROENZYME FORM OF HUMAN UROKINASE.

Wun TC, Ossowski L, Reich E
Rockefeller Univ., New York, NY, 10021
J Biol Chem; 257(12):7262-7268 1982

A culture of the human epidermoid carcinoma Hep 3 produces a plasminogen activator of Mr = 53,000 which we have purified to apparent homogeneity from serum-free conditioned medium by the combination of immunoaffinity chromatography and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The highly purified protein has the following properties: (1) It is indistinguishable from urinary urokinase in electrophoretic mobility, in immunodiffusion, and in autoradiographically visualized tryptic peptide maps obtained from the 125I-labeled proteins. (2) The HEp 3 protein differs from urinary urokinase in the following respects: (a) although the apparent mol wt of the two are identical (Mr = 53,000), the urinary enzyme consists of two polypeptide chains, whereas the HEp 3 protein is a single chain form. (b) Urinary urokinase can be labeled easily by incubation with radioactive diisopropylfluorophosphate but the HEp 3 protein cannot. (c) When assayed by the hydrolysis of a synthetic chromogenic peptide substrate, the Hep 3 enzyme has less than 1% of the catalytic activity of urinary urokinase. (3) On controlled exposure to plasmin, the HEp 3 protein is converted to an active enzyme that is identical with urinary urokinase in mol wt, polypeptide chain composition, diisopropylfluorophosphate labeling, and specific catalytic activity. We conclude that the HEp 3 protein is a proenzyme that can be converted to active two-chain urokinase by plasmin, probably by a single proteolytic nick in the polypeptide chain. (Author abstract) (26 Refs)

26. PRIMARY STRUCTURE OF A HUMAN LAMBDA-CHAIN (WEIR) OF THE MCG TYPE.

Jabusch JR, Deutsch HF
Dept. Physiological Chemistry, Univ. Wisconsin, Madison, WI
Mol Immunol; 19(7):901-906 1982

The amino acid sequence of the Bence-Jones protein Weir has been determined. This lambdaII protein is of the Mcg type. Since a mixed dimer of Weir and Mcg can be prepared in a form suitable for crystallographic analysis it was of interest to compare the sequences of these two lambda-chains. A total of 37 differences, which include one previously determined constant-region substitution, have been found. (Author abstract) (22 Refs)

27. THE INTERACTION OF MOUSE MYELOMA IMMUNOGLOBULIN S15 WITH NEGATIVELY CHARGED POLYSACCHARIDE ANTIGENS.

Manjula BN, Potter M, Glaudemans CP
(c/o Glaudemans), NCI, NIH, Bethesda, MD, 20205
Mol Immunol; 19(7):913-923 1982

A murine BALB/c IgG2a (lambda3) myeloma immunoglobulin SAPC-15 with binding activity for negatively charged polysaccharides has been purified by affinity chromatography, and its interaction with heparin and various other polyanionic antigens has been studied. The antigen-binding activity has been demonstrated to reside in the Fab part of the immunoglobulin. The S15 myeloma protein in 0.05 M Tris buffer at pH 7.4 precipitated dextran sulfate, heparin, chondroitin sulfate A, B and C, hyaluronic acid, H influenzae Type b polysaccharide, calf thymus DNA, Klebsiella polysaccharide K63 and poly-L-glutamic acid. Of these antigens only dextran sulfate was precipitated in 0.01 M phosphate buffered saline (0.15 M), pH 7.4. The pepsin S15 Fab fragment did not precipitate with any of these antigens. The intrinsic tryptophanyl fluorescence of S15 was changed maximally by the addition of heparin, and the binding affinity of the immunoglobulin for this antigen was high (greater than 10(6) L/M). S15 may resemble antibody molecules that react with antigens under non-physiological conditions or in pathological conditions or in the external environment as in the lumen of the gut. All the above interactions of S15 with antigens persisted in 0.05 M Tris buffer made physiologically isotonic by the addition of sucrose, and S15 could thus be used to identify these antigens on cell surfaces. (Author abstract) (49 Refs)

28. PHYSICOCHEMICAL AND IMMUNOCHEMICAL CHARACTERISTICS OF HEAVY CHAINS OF CANCER-SPECIFIC IGG.

Sen'ko LN, Protsenko BA, Korotkoruchko VP
A. V. Palladin Inst. Biochemistry, Kiev, USSR
Ukr Biokhim Zh; 54(3):243-248 1982

Results of physicochemical and immunochemical analysis of IgG isolated from sera of myeloma G patients are reported. Thin layer gel filtration showed significant decreases in mol wt of heavy chains of cancer-specific IgG (43,000 daltons, compared with 49,500-51,000 daltons for IgG of healthy controls). The decrease in mol wt was associated with a significant decrease in number of amino acid residues. Double immunodiffusion technique showed that rabbit antiserum to the cancer-specific IgG precipitated gamma chains of the cancer-specific IgG but not gamma chains of IgG from healthy controls. These findings indicated that heavy chains of cancer-specific IgG contained antigenic determinants. Analysis of cyanogen bromide fragments showed changes in content of tripeptides. It was concluded that the differences in the physicochemical properties of the cancer-specific IgG are associated with changes in primary structure. (29 Refs) (In RUS)

29. COLLAGEN SYNTHESIS STIMULATING ACTIVITIES PRODUCED BY RAT MAMMARY ADENOCARCINOMAS (MEETING ABSTRACT).

Bano M, Zwiebel J, Salomon D, Kidwell WR
Laboratory of Pathophysiology, National Cancer Institute, Bethesda, MD, 20205
Proc Am Assoc Cancer Res; 23:901 1982

Mammary adenocarcinomas synthesize type IV collagen and regress if synthesis of this protein is selectively blocked. These tumors apparently possess a constitutive mechanism for promoting collagen formation since they produce factors (MTF) that differentially stimulate the incorporation of 14C-proline into collagenase sensitive proteins by 3-10 fold. In cultures of normal rat mammary epithelium, MTF stimulates total protein labeling by 30% and collagen labeling by 300%. Stimulation is seen as early as three hrs and is apparently the result of increased collagen synthesis rates since pulse-chase studies indicate that MTF does not affect the rate of collagen degradation. The tumor cells that produce MTF are not responsive to exogenously added MTF indicating that MTF production is adequate for maximal rates of collagen synthesis by these cells. MTF may act via the epidermal growth factor (EGF) receptors on cells since a receptorless derivative of 3T3 cells is unresponsive to MTF. The action of EGF on normal mammary cells which possess EGF receptors results in only a 40% differential stimulation of collagen synthesis; ie, MTF is about 8 fold more potent than an optimal concentration of EGF (2.5 ng/ml). Crude MTF prepared from tumors by acid-ethanol extraction has been further purified by gel filtration and cation exchange chromatography. Both methods indicate that there are two active species of MTF, one with a molecular weight of 65,000 and the other about 1/10 this size. Preparative isoelectric focusing indicates that the active fractions have a pI ranging from 5.7 to 6.3. The activities are inactivated by boiling for 5 min and thus are probably proteins. MTF may play an important role in supporting the growth of mammary adenocarcinomas. (no Refs)

30. PURIFICATION AND CHARACTERIZATION OF A COLLAGENOUS PROTEIN SECRETED BY A MURINE TERATOCARCINOMA-DERIVED CELL LINE.

Ko CY, Priest RE
Dept. Pathology, Lab. Medicine, Emory Univ., Atlanta, GA, 30322
Biochim Biophys Acta; 720(3):288-294 1982

A collagenous protein could be precipitated by (NH4)2SO4 from the culture medium of a murine teratocarcinoma-derived cell line. Further purification of this protein was achieved by combining DEAE-cellulose chromatography with either CM-cellulose or molecular sieve chromatography. The collagenous polypeptides had subunit mol wt of 160,000, if determined by molecular sieve chromatography, or 190,000, if determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and they are not linked by disulphide bridges. Amino acid composition of this collagen is similar to that of a murine Type IV collagen isolated from EHS sarcoma. The most prominent peptides resulting from cleavage of the protein by CNBr had estimated mol wt of 25,000, 23,000, 11,700 and 9,400. Pepsin treatment of this collagen under non-denaturing conditions produced three major fragments having molecular wt of 70,000, 45,000 and 43,000. We conclude that the collagen secreted by the murine teratocarcinoma-derived cell culture is a Type IV basement membrane collagen. Therefore, this culture system should provide a continuous source of Type IV collagen, which may be used to study the interaction of this collagen with other basement membrane components. (Author abstract) (22 Refs)

31. CRITICAL REMARKS ON THE ROLE OF COLLAGENOLYTIC ENZYMES IN CARCINOMAS OF THE HEAD AND NECK.

Ganzer U, Ebbers J
Dept. Otorhinolaryngology, Univ. Dusseldorf, Moorenstrasse 5, D-4000 Dusseldorf, W. Germany
Arch Otorhinolaryngol; 234(3):263-266 1982

Collagenolytic activity of tissues was studied by two methods. In the collagen gel method, small tissue specimens were incubated on a reconstituted collagen gel. After 4 days at 37 C, the size of a clear concentric zone was related to the enzyme activity. The other method determined change in viscosity after incubation of a tissue homogenate. The collagenase activity of eight squamous cell carcinomas of the head and neck were investigated as was that of xenografts of the tumors in nude mice. Incubation of the original tumor specimens on collagen gels produced only small transparent areas. Specimens from the proliferating zone of xenografts produced greater zones of lysis, while tissue from the necrotic parts of the xenografts induced the largest areas of transparency. After incubation of both original and denatured tumor, a 30% decrease in viscosity occurred within the first 2 hr, and about 50% decrease after 24 hr. The viscosity of pure collagen decreased 50% within 2 hr and 30% after 24 hr. A hypothetical concept, including the results of other authors, is developed concerning the role of collagenase in infiltrative growth of malignant tumors. (5 Refs)

32. SULFATED GLYCOSAMINOGLYCAN AND COLLAGEN PATTERNS IN PARIETAL YOLK SAC CARCINOMA (PYSC).

De Fatima Bonaldo M, Cassaro-Strunz CM, Machado-Santelli G
Fundacao 'Centro de Pesquisa de Oncologia', P.B. 11.490, Sao Paulo, Brazil
Cell Differ; 11(2):99-106 1982

Collagen and sulfated glycosaminoglycans (GAG) were analyzed in mouse parietal yolk sac carcinoma (PYSC) in vivo and in vitro, to correlate the substances with basement membrane synthesis. The collagen-like polypeptide patterns obtained from the incubation medium material of PYSC are presented. Two polypeptide components were found to migrate as type I collagens, and the analysis of the alpha1/alpha2 ratios ranged from approx 2-3. The change observed in the pattern of GAG sulfate in the solid tumor, inverting the proportion of heparan sulfate and chondroitin sulfate B, could be a consequence of the cellular differentiation that was observed at a growth rate which occurred during development of the tumor. (32 Refs)

33. ALTERED GLYCOSYLATION OF CHORIOGONADOTROPINS FROM JEG-3 CELL CULTURE (MEETING ABSTRACT).

Kellen JA, Malkin DG, Chan AW, Malkin A
Department of Clinical Biochemistry, Sunnybrook Medical Center, University of Toronto, Toronto M4N 3M5, Canada
Proc Am Assoc Cancer Res; 23:928 1982

Plant lectins are useful probes for studying protein glycosylation, and can be used to indicate chemical alterations in cell surface architecture and products from malignant cells. The JEG-3 model used in this study is derived from a human trophoblastic tumor and is known to actively secrete choriogonadotropins (CG) in cell culture. We studied binding of the secreted CG to Concanavalin A (Con A) and Ricinus conjugated Sepharose 4B columns, in comparison with the product of normal 3rd trimester placental cells and after a pulse of gonadoliberin (LHRF, 1 ug/ml), dbCAMP (lmM), Con A (100 ug/ml) and Wheat Germ lectin (100 ug/ml), respectively. CG was determined by radioimmunoassay (RIA) using an antibody to the beta subunit. Our results indicate that while the CG released by normal trophoblast cells into the culture medium is 90% Con A-bound, up to 40% of CG from JEG-3 is not bound to this lectin; however, 25% of this 'free' fraction binds to Ricinus and is therefore glycosylated, containing D-Gal moieties. Exposure of JEG-3 cultures to LHRF, Con A, and dbCAMP increased CG secretion, but did not alter the proportions of CG binding to these lectins. Wheat Germ agglutinin inhibited cell growth. We conclude that malignant cells of trophoblastic origin synthesize a substantial part of CG with an altered pattern of glycosylation. This pattern is characteristic and constant, it does not change with increase or decrease of CG synthesis in the cell culture nor with numerous passages. (1 Ref)

34. GENERATION OF A COMPLEMENT-DERIVED CHEMOTACTIC FACTOR FOR TUMOR CELLS IN EXPERIMENTALLY INDUCED PERITONEAL EXUDATES AND ITS EFFECT ON THE LOCAL METASTASIS OF CIRCULATING TUMOR CELLS.

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Am J Pathol; 108(1):112-118 1982

A chemotactic factor for tumor cells was found in inflammatory exudate fluids induced by giving intraperitoneal injections of glycogen to Sprague-Dawley rats. The quantity of chemotactic activity and the period of time during which it could be detected correlated with the inflammatory reaction, measured by the cellular composition of the exudates and their content of protein and lysosomal enzymes. In gel filtration the chemotactic factor behaved mainly as a molecule having a molecular weight of approx 6,000 daltons. Its biologic activity was blocked by antiserums directed against C5 but not by antiserums against C3 or C4. In these two respects, the factor generated in vivo has the same properties as a previously described chemotactic factor that can be generated in vivo by proteolysis of purified C5 or C5a. Chemotactic activity was not detected in the glycogen-induced peritoneal exudates of rats depleted of serum complement by cobra venom factor. Intravenously injected Walker tumor cells arrested and formed metastases in the mesenteries of rats with peritonitis in greater numbers than in normal controls, animals depleted of complement during the experimental period, or animals given intraperitoneal injections of the vasopermeability agent, histamine. The growth of tumor cells in vitro was not promoted by peritoneal exudate fluids, nor was the number of metastases in vivo greater than in negative controls, in animals in which peritonitis was induced 24 hr after the intravenous injection of tumor cells. It is argued that chemotactic mechanisms can contribute to the formation of metastases at sites of tissue injury. (Author abstract) (20 Refs)

35. LAMININ INHIBITS ATTACHMENT OF NORMAL AND TRANSFORMED MURINE MACROPHAGES (MEETING ABSTRACT).

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Proc Am Assoc Cancer Res; 23:172 1982

Metastatic tumor cells must adhere to the capillary basement membrane of distant organs before extravasation. The importance of this step has prompted studies on the regulation of adhesive characteristics of neoplastic cells. Laminin, a basement membrane glycoprotein, has been shown to serve as an attachment factor for some metastatic tumor cells. In the present study the adhesive behavior of a highly metastatic murine tumor, M5076, was investigated in vitro in the presence and absence of purified exogenous laminin. The presence of laminin in adhesion assays stimulated the attachment of normal embryo fibroblasts, murine melanoma cells and fibrosarcoma cells to collagen IV and tissue culture plastic. In contrast laminin inhibited the attachment of M5076 reticulum sarcoma cells to these same substrates. This inhibitory effect appeared to be related to the macrophage origin of the M5076 tumor as indicated by the finding that the attachment of normal murine peritoneal exudate cells and WEHI-3, another macrophage tumor, was similarly inhibited. Furthermore, the effect was dose dependent, was reversible, and had no effect on the viability or functional capacity of the treated cells. This activity was a consequence of laminin binding to the substrates rather than to the cells, as demonstrated by separate preincubation studies. These results show that regulation of the adherence behavior of metastatic tumor cells is not universal but depends on the origin of the tumor. (no Refs)

36. CATABOLISM AND EXCRETION OF FIBRINOPEPTIDE-A.

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Veterans Admin. Medical Center, 111G-JB, St. Louis, MO, 63125
Blood; 60(1):148-156 1982

Mean urinary excretion of fibrinopeptide-A (FPA) in 51 normal individuals ranging in age from 7 to 92 yr was 1.67 +- 0.1 (SE) ug/24 hr with no significant differences related to age or sex, though children tended to excrete less FPA. Calculation suggests that FPA urinary excretion represents 0.2%-0.5% of total FPA released from fibrinogen/24 hr. Urinary FPA eluted from gel permeation columns in the same position as 125I-FPA. 125I-FPA (human) was cleared from dog plasma with a half-life of 3.9 min, and 0.5% of the total dose could be accounted for in the urine over a 3-hr period. FPA assay in paired plasma and 24-hr urine samples from 15 normal individuals, 16 patients with malignancy, 3 patients with other disease, and 5 patients with severe burns (several samples from each) demonstrated significant correlation between plasma FPA concentration and 24-hr urinary FPA excretion (p less than 0.001). Mean plasma FPA in patients with malignancy was 5.9 ng/ml with a urinary excretion of 8.7 ug/24 hr, in the burn patients 21.6 ng/ml plasma with 32.5 ug FPA/24 hr excreted in the urine; all values were significantly higher than normal (p less than 0.001). Mean urinary excretion of FPA in eight myocardial infarction patients was 5.35 ug/24 hr (p less than 0.01). Thrombin increasable FPA was found in both bentonite-adsorbed plasma and in urine. Thrombin increasable FPA averaged 20% in normals, 50% in patients with malignancy, 100% in burn patients, and was variable in myocardial infarction patients, some of whom had a several-fold increase on Day 1 and on subsequent days increases of 20%-50%. Free FPA and thrombin increasable FPA could be distinguished by gel permeation chromatography. (Author abstract) (14 Refs)

37. FIBRIN DEPOSITS IN HODGKIN'S DISEASE.

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Am J Pathol; 108(1):119-129 1982

Fibrin deposits were observed in the involved lymph nodes and/or spleens of 15 patients with Hodgkin's disease by specific immunofluorescence and by electron microscopy. Two basic patterns of fibrin deposition were observed: (1) intercellular deposits, chiefly associated with nonneoplastic-appearing lymphoid cells and (2) deposits associated with the collagen fibers of young connective tissue. In addition, coarse fibrin deposits were observed in areas of necrosis, presumably a nonspecific finding. Fibronectin was also observed in intercellular areas, but staining was less intense than for fibrin. Fibrin deposits were also observed in 3 of 6 cases on non-Hodgkin's lymphoma, indicating that the finding is not an exclusive feature of Hodgkin's disease. The pathogenesis and possible significance of fibrin deposition in Hodgkin's disease are related to earlier observations of activation of the coagulation system in neoplasia and cell-mediated immunity and to the possible role of fibrin, fibronectin, and their breakdown products in angiogenesis and fibroplasia. (Author abstract) (38 Refs)

38. ISOLATION AND PURIFICATION OF A DNA-BINDING PROTEIN THAT INHIBITS DNA DEGRATION BY BLEOMYCIN AND IS DECREASED IN SERA OF CANCER PATIENTS.

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University of Houston, Houston, TX
Diss Abstr Int [B]; 42(8):3232-B 1982

Using the PM2 DNA fluorescence assay to guide its fractionation, the Bleomycin Inhibitor Protein has been purified to homogeneity by ammonium sulfate precipitation, DEAE (diethylaminoethyl)-cellulose column chromatography and DNA-cellulose affinity column chromatography. Two-dimensional isoelectric focusing gel electrophoresis showed a homogenous protein having a molecular weight of 64,000 and a pI of 5.9. Amino acid composition analyses revealed an acidic to basic residue ratio of 1.2. The NH(2)-terminus amino acid was lysine as determined by microdansylation and polyamide thin layer chromatography. The inhibition was abolished by pronase but not by RNase or DNase treatment. DNA-binding was demonstrated by gel filtration column chromatography, agrose gel electrophoresis, fluorescence quenching and circular dichroism studies. The bleomycin inhibitor has biochemical properties similar to superoxide dismutase (SOD). The SOD-like activity was found to be decreased in the sera of cancer patients that had been shown to contain low levels of bleomycin inhibitor activity by the PM2 DNA fluorescence assay. Some differences between SOD and the bleomycin inhibitor have been discerned. Both proteins bind PM2 and inhibit the bleomycin-induced degradation but whereas the bleomycin inhibitor lessens the mobility of form I DNA on agarose gels, SOD does not have the same effect. Also, the bleomycin inhibitor is more susceptible to inactivation by heat than SOD. Lastly, the two proteins contain different NH(2)-terminal amino acids. The bleomycin inhibitor has potential as a biological marker for cancer. (no Refs)

BINDING/ RECEPTOR PROTEINS

39. EVIDENCE THAT GALACTOSYLTRANSFERASE IS A SURFACE RECEPTOR FOR POLY(N)-ACETYLLACTOSAMINE GLYCOCONJUGATES ON EMBRYONAL CARCINOMA CELLS.

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J Biol Chem; 257(12):6871-6878 1982

Cell surface glycosyltransferases are thought to participate in a variety of cellular interactions, but their specific glycoside acceptors have received little attention. In this paper, poly(N)-acetyllactosamine glycoconjugates are shown to be the endogenous substrates for embryonal carcinoma (EC) cell surface galactosyltransferases. All controls have been performed to ensure a surface localization for the galactosyltransferase activity. The galactosylated product(s) is relatively insoluble in organic solvents, is larger than conventional glycopeptides following pronase digestion, and is highly sensitive to endo-beta-galactosidase degradation. Solubilized polylactosaminyl glycoconjugates serve as competitive exogenous acceptors for the surface galactosyltransferase. In addition, the endogenous galactosyl acceptor(s) reacts with antiserum directed against EC cell poly(N)-acetyllactosamines. Anti-EC antiserum inhibits galactosylation of endogenous acceptors, simultaneously stimulates galactosylation of an exogenous acceptor, and immunoprecipitates 74% of the reaction product. Differentiated EC cells no longer react with anti-EC antiserum and no longer show anti-EC antiserum effects on surface galactosyltransferase activity. Interestingly, forced galactosylation with UDPGal releases polylactosaminyl substrates from the cell surface. In the absence of UDPGal, glycoconjugate release is dramatically reduced. GDPMan cannot substitute for UDPGal, and a galactosyltransferase inhibitor prevents glycoside release from the cell surface. Thus, surface galactosyltransferase preferentially binds poly(N)-acetyllactosamine glycoconjugates and serves as at least one of their surface receptors on EC cells. (Author abstract) (27 Refs)

40. THYROTROPIN RELEASING HORMONE RECEPTORS IN REGULATION OF PROLACTIN GENE EXPRESSION IN GH CELLS.

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Biochem Biophys Res Commun; 106(3):811-817 1982

The thyrotropin releasing hormone (TRH) receptor-modulated regulation of prolactin (PRL) gene expression was studied in three GH cell strains (rat pituitary tumor cells in culture). The three strains synthesize different amounts of PRL and exhibit different responses to TRH. Binding affinities and the number of receptors/cell were studied and the effect of epidermal growth factor on PRL gene expression in the different cell strains was explored. One strain (GH4C1) responded to TRH by stimulating PRL production while the hybrid strain (928-9b) and the bromodeoxyuridine-resistant strain (F1BGH12C1) did not. When binding of 3H-TRH was studied as a function of TRH concentration, the affinities of TRH to its specific receptors were similar in both GH4C1 and 928-9b cells. However, the number of TRH receptors/cell was significantly different in the two strains; GH4C1 cells had a max number of specific binding sites of 1.05 x 10(5)/cell, while 928-9b cells had a max number of 0.16 x 10(5)/cell. Thus, in the two strains, the TRH-responsive GH4C1 cells and the TRH-nonresponsive cells, the total number of TRH binding sites per cell was much less in the TRH-nonresponsive cell line. The inadequate number of TRH receptors in 928-9b cells may be responsible for the nonresponsive property of the hybrid cells to TRH and a possible role of the number of specific receptors in the regulation of the peptide hormone action is suggested. (11 Refs)

41. REGULATION OF INSULIN RECEPTORS IN ERYTHROID CELLS.

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Metabolism; 31(7):728-732 1982

The effect of exogenous insulin on receptor concentration and affinity of cells of the erythroid series was studied using murine Friend erythroleukema (FEL) cells and rabbit reticulocytes. Undifferentiated and differentiated FEL cells, models for erythroblasts and normoblasts, conducted both active protein synthesis and messenger RNA synthesis; reticulocytes were incapable of mRNA synthesis, but were able to synthesize protein, and mature erythrocytes could synthesize neither. Each type of cell was placed into Temin's medium containing 15% fetal calf serum with or without 10 nanoM insulin for 18 hr at 37 C. Decreased binding of (125-I)-iodoinsulin tracer following insulin exposure by erythroblasts, normoblasts, and reticulocytes was due to changed receptor concentrations. Insulin treatment did not significantly alter receptor affinity characteristics for any cell type except the erythrocyte. Basal values for receptor concentration were 30,000 sites/cell for erythroblast, 10,000 sites/cell for normoblast, 190 sites/cell for reticulocyte, and 45 sites/cell for erythrocyte. The ability to 'down-regulate' was lost as follows: erythroblasts down-regulated by 60%, normoblasts by 43%, and reticulocytes by 30%; erythrocytes did not down-regulate. These results are consistent with the hypothesis that protein synthesis is needed for insulin receptor down-regulation. The possibility that mature erythrocytes cannot reduce insulin receptor concentration in response to hyperinsulinemia suggests that utilizing erythrocytes as models of total body receptor concentrations may only apply to chronic changes. (29 Refs)

42. VARIATIONS IN THE LEVELS OF ESTROGEN RECEPTORS IN PROLACTIN PRODUCING PITUITARY TUMOR CELLS.

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Dept. Biochemistry, Univ. Missouri Sch. Medicine, Columbia, MO, 65212
J Steroid Biochem; 16(5):633-638 1982

The binding of (3H)-17beta-estradiol in the cytoplasmic fraction of MtTW10 rat pituitary tumor cells was investigated. The capacity to bind estrogen decreased with time in culture but was not accompanied by a change in the affinity of the receptor for estrogen. When the binding of (3H)-17beta-estradiol in the 105,000 gravity cytosol of GH3 rat tumor cells was measured, the dissociation constant was found to be 2.1 X 10(-10) M and the receptor concentration was equivalent to 282 femtomoles estradiol/mg protein. Receptor concentration of the low speed supernatant of GH3 cells was 70 femtomoles estradiol/mg protein. This lower value was due to the higher amount of non-receptor protein present in this supernatant. Estrogen receptors in the MtTW10 cells were very similar to those of other rat pituitary tumors. Like normal pituitary cells, both the GH3 and the MtTW10 cells contained a receptor in the cytosol with a sedimentation constant of 8 svedberg units. Receptor could bind estrogen even in the presence of high concentrations of digitonin. The decreased binding of estradiol observed in the cytoplasmic fraction of MtTW10 cells during tumor aging was due to a decrease in the number of binding sites rather than to a change in the affinity of the receptor for estrogen. Decreased levels of receptors could be due to a decrease in the av rate of synthesis of receptors or to a complete cessation of synthesis of receptors in cells which overgrew in culture. Human pituitary tumor cells (18-54) completely lacked high affinity estrogen receptors, whether or not serum was present in the culture medium. (13 Refs)

43. TROPHOBLASTIC CELLS OF THE HYDATIDIFORM MOLE CONTAIN A B1-SUBTYPE ADRENERGIC RECEPTOR.

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J Clin Endocrinol Metab; 55(2):341-346 1982

Both beta-1 and beta2-adrenergic receptors have been previously described in normal human placental homogenates; the cells upon whose surface membranes these receptors reside have not been identified. In order to show that a beta1-adrenergic receptor is present on trophoblastic cells, the cells which mediate maternal-fetal transport and produce placental hormones, beta-adrenergic receptors were demonstrated in membrane fractions of human hydatidiform mole. Microscopic sections of the mole samples used demonstrated edematous villi lined by trophoblastic cells with minimal nontrophoblastic (stromal or vascular) contamination compared with placenta. (-)-[3H]Dihydroalprenolol [(-)-[3H]DHA]binding to molar membranes was reversible and saturable to a single class of sites (Kd = 0.97 +-0.12 nM; n = 7; maximum binding capacity, 72.9 +-6.4 fmol/mg protein). (-)-[3H]DHA binding was associated with catecholamine-stimulated adenylate cyclase activity. Agonist competition for the molar beta-adrenergic receptor showed the order of potency to be (-)isoproterenol much greater than norepinephrine = epinephrine, characteristic of a beta1-adrenergic receptor subtype. Competition for (-)-[3H] DHA binding to trophoblastic membranes by the beta-adrenergic receptor subtype-specific agents metoprolol (beta1 selective) and zinterol (beta2 selective) was also characteristic of a homogenous subtype of beta1-adrenergic receptors. Because beta1-adrenergic receptors alone were seen on trophoblast cells, the beta2-adrenergic receptor in placenta must reside on nontrophoblastic elements (stromal or vascular endothelium). No differences in beta-adrenergic receptor binding were seen related with ploidy (2 or 3 N), the presence or absence of a fetus, or the progression of the mole to choriocarcinoma. Two choriocarcinoma cell lines, BeWo and JEG-3, however, showed no specific (-)-[3H]DHA binding. Human trophoblast contains beta1-adrenergic receptors coupled to catecholamine-sensitive adenylate cyclase, supporting a role for catecholamines in the regulation of placental metabolism. (Author abstract) (26 Refs)

44. VITAMIN A AND RETINOL-BINDING PROTEIN IN PATIENTS WITH MYELOMATOSIS AND CANCER OF EPITHELIAL ORIGIN.

Basu TK, Rowlands L, Jones L, Kohn J
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Eur J Cancer Clin Oncol; 18(4):339-342 1982

Serum vitamin A and retinol-binding protein (RBP) concentrations were measured in patients comprising 53 myeloma and 28 epithelial cell cancer cases. Vitamin A levels in these patients were found to be significantly lower than those in the 30 healthy subjects, the effect being more marked in the patients with cancer of epithelial origin. The serum concentrations of retinol-binding protein (RBP) fell in parallel with vitamin A in the epithelial cancer patients, while the RBP concentrations remained unaffected in the patients with myeloma, suggesting that the underlying factor for resulting low vitamin A levels may be different in these two groups of patients. (Author abstract) (22 Refs)

45. CHARACTERIZATION OF THE RECEPTORS FOR IGE ON MEMBRANES ISOLATED FROM RAT BASOPHILIC LEUKEMIA CELLS.

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Mol Immunol; 19(7):925-941 1982

Mast cells, basophils and a tumor analog, rat basophilic leukemia cells, have a surface receptor for immunoglobulin E (IgE). Membranes from such cells should be highly useful for the study of how the alpha- and beta-subunits of the receptor interact with the plasma membrane and function therein. Membranes were prepared from rat basophilic leukemia cells. The cells were reacted with radioiodinated IgE to saturate a fraction of their receptors and the radiolabel was used as a surface marker. When isolated from sucrose gradients, 40 +-10%

yields of plasma membranes were obtained and these were typically purified 13-fold with regard to cellular proteins. Unoccupied receptors for IgE were almost all recovered (as assessed on membranes solubilized with detergent) as were surface-labeled proteins in general. The membrane-bound IgE appeared fully exposed as assessed with anti-IgE antibodies but surprisingly 70-80% of the unoccupied receptors present were unable to bind IgE when unsolubilized vesicles were tested. By electron microscopy the preparations appeared free of recognizable intracellular organelles. Though some of the membranes appeared multivesicular many showed large breaks in continuity. Assessment of permeability, carried out on dextran gradients, indicated that a significant number (greater than or equal to 50%) of the IgE-carrying membranes are not sealed. It is therefore most likely that both external and cytoplasmic aspects of the plasma membrane were exposed in many of the vesicles. Treatment of such membrane vesicles with trypsin or papain did not alter the apparent mol wt of the alpha-chain of the receptor or its binding activity for IgE. Additionally, when the vesicles were examined by enzyme-catalyzed iodination, no labeling of the receptor was observed under conditions where it is not labeled on intact cells (ie in the presence of IgE). These results suggest that either the alpha-chain of the receptor does not penetrate the membrane or that it cannot be modified by the particular probes we used. (Author abstract) (34 Refs)

46. THE TCDD RECEPTOR IN RAT INTESTINAL MUCOSA AND ITS POSSIBLE DIETARY LIGANDS.

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Nutr Cancer; 3(3):134-144 1982

Induction of aryl hydrocarbon hydroxylase (AHH) by polycyclic aromatic hydrocarbons and other inducers such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is known to occur following binding of the inducer to a soluble receptor protein similar to steroid hormone receptors. This receptor is usually called the TCDD receptor, since TCDD has the highest affinity of all known ligands for the receptor. In the present paper, a receptor for TCDD in cytosol from rat intestinal mucosa has been studied, using isoelectric focusing in polyacrylamide gel. This receptor's biochemical properties were found to be similar to those of the TCDD-receptor in rat liver cytosol. The dissociation constant (Kd) of the 3H-TCDD-receptor complex in rat intestinal mucosa was 0.7-3.1 nM, and it was present at a concentration of 70-80 fmol/mg protein. Starvation did not significantly increase the receptor level. The affinities of some potential dietary ligands for the TCDD receptor in rat intestinal mucosa were also studied. Indole-3-carbinol had 1/2,600 of the affinity of TCDD for the receptor protein. Butylated hydroxyanisole (BHA), transstilbene oxide and quercetinpentamethylether competed even more weakly with 3H-TCDD for binding to the receptor. The biological significance of the occurrence of low-affinity ligands of dietary origin for the TCDD receptor is uncertain at the present time. (Author abstract) (37 Refs)

47. SPECIFIC CYTOSOL BINDING PROTEINS FOR 25(OH)D3 AND 1.25(OH)2D3 IN HUMAN BREAST CANCERS.

Nakata M, Tsuboi S, Takenaka M, Konishi Y, Kawashima R, Tomita M, Nakagawa T, Iwamura N, Fujita T
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Endocrinol Jpn; 28(6):741-746 1981

Tissue from 30 breast cancers obtained at mastectomy was investigated for the presence of binding proteins for 1.25(OH)2D3 and 25(OH)D3. Specific, high affinity cytosol binding proteins for both compounds were found. Sucrose density sedimentation analysis demonstrated the presence of a 3.7 Svedberg (S) protein in 1.25(OH)2D3 and a protein of approx 6.0 S in 25(OH)D3. Scatchard analysis demonstrated a dissociation constant (Kd) for 1.25(OH)2D3 ranging from 0.1 x 10(-11) to 7.1 x 10(-11) and a binding capacity of 0.94-26.0 femtomoles/mg protein of 1.25(OH)2D3. The ability of several structural analogs to inhibit binding of [3H]1.25(OH)2D3 to the binding protein was studied. The order of effectiveness of competition was 1.25(OH)2D3 much greater than 25(OH)D3 greater than 1alpha(OH)D3, 24,25(OH)2D3 greater than D3 much greater than estradiol-17beta, dexamethasone, DHT, and progesterone. The competition study for 25(OH)D3 revealed relative specificities of 24,25(OH)2D3 much greater than 1.25(OH)2D3 greater than D3 greater than 1alpha(OH)D3. Twenty-eight of the 30 breast cancers were positive for 1.25(OH)2D3 receptor. (15 Refs)

MEMBRANE/ SURFACE PROTEINS

48. A NEW MARKER FOR HUMAN CANCER CELLS. 1. THE CA ANTIGEN AND THE CA1 ANTIBODY.

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Lancet; 2(8288):1-6 1982

In a search for antibodies that might distinguish between malignant and non-malignant cells a panel of matched pairs of hybrid cells produced by fusion of diploid fibroblasts with malignant cells originating from a cervical carcinoma was used as a screen. Each pair consisted of a hybrid in which malignancy was suppressed and a malignant segregant derived from this hybrid. A monoclonal antibody, designated Ca1, was found that discriminated absolutely between the hybrids in which malignancy was suppressed and the malignant segregants. This antibody detected an antigen present in the cell membranes of a wide variety of malignant human cell lines but not of diploid human cell strains. The antigen was found in very low concentrations, if at all, in homogenates of normal adult or fetal tissues. It could be immunoprecipitated by the Ca1 antibody from extracts of malignant cells but not from extracts of non-malignant cells. After reduction, the immunoprecipitated antigen separated in sodium dodecyl sulfate acrylamide gels as two bands with approx molecular masses of 390,000 and 350,000. These two components had the properties of glycoproteins with a high carbohydrate content; both bound the Ca1 antibody. The antigenic determinant resisted boiling at 100 C and extraction by a range of organic solvents. The binding of the Ca1 antibody to the antigen was substantially reduced by treatment of the antigen with neuraminidase, and the antigenic determinant was largely destroyed by certain endoglycosidases and by extensive proteolysis. Pending its further characterization, this antigen has been called the Ca antigen. (Author abstract) (24 Refs)

49. AN IMMUNOHISTOCHEMICAL 5'-NUCLEOTIDE PHOSPHODIESTERASE METHOD FOR THE DIAGNOSIS OF LIVER CANCER IN HUMAN LIVER BIOPSY (MEETING ABSTRACT).

Tsou KC, Lo KW, Witzleben CL, Suganuma T
Department of Surgical Research, University of Pennsylvania, Philadelphia, PA, 19104
Proc Am Assoc Cancer Res; 23:67 1982

Serum 5'-nucleotide phosphodiesterase isozyme (5'-NPD-V) has been reported as a useful test for liver cancer. In the course of the study of the development of the isozyme, a pure human 5'-nucleotide phosphodiesterase has been isolated from normal liver specimen with diethylaminoethanol (DEAE)-Sephacel or concanavalin A (CorA)-Sepharose 5B chromatography. Anti-sera of this enzyme was prepared from rabbits and tested in an immunohistochemical procedure, using peroxidase-antiperoxidase (PAP) method, and diaminobenzidine (brown), as a substrate. To avoid hemosiderin in liver, a new method developed by us using 4-dimethylamino-1-naphthylamine and 2,5-dihydroxy-naphthalene (blue) were also used. The tumor cells in liver stain 2 to 3 times darker than the surrounding normal liver in all six cases hepatocellular carcinoma examined and one case of metastasized liver. Carcinoembryonic antigen stains also weakly and alpha fetoprotein faintly or none in the same tumor area. In cirrhotic area this enzyme is present but not elevated. Orcein stain for HBsAg (Shikata) also found positive in tumor area 2/3 patients whose sera was HBsAg negative, and negative in 2/2 patients whose sera was HBsAg positive. These findings further support the suggestion that 5'-NPD is a growth related enzyme. This study supports the oncofetal expression of the 5'-NPD in tumor and the heterogeneity of antigen expression in liver cancer. (no Refs)

50. ENZYMATIC PROPERTIES IN PERICELLULAR MEMBRANES OF LEUKEMIC CELLS.

Losa G
Lab. Cellular Pathology, Ticino Inst. Pathology, Locarno, Switzerland
Adv Exp Med Biol; 145:377-384 1982

Enzyme characteristics of the plasma membrane were analyzed biochemically in cell populations isolated from the peripheral blood of leukemic patients to distinguish the various types of acute leukemias. Very low enzyme activities were generally recorded in cells of both non B/non T lymphoblastic and T leukemias. Differences in the activity of some enzymes were sufficient to allow a clear-cut differentiation of the two leukemic cell populations. In lymphoblastic leukemia high 5'-nucleotidase activity was measured with a mean level of approx 45 nanomole (nmol)/hr/10(6) cells. In some patients, the activity reached a mean value of 230 nmol/hr/10(6) cells, suggesting an earlier differentiation stage of the corresponding leukemic cells. Compared to the control mononuclear cell population, moderate decreased activities were measured for 5'-nucleotidase, alkaline phosphomonoesterase, and gamma-glutamyltranspeptidase. The activity of 5'-phosphodiesterase was significantly high in approx 40% of the patients while in the remainder it was practically undetectable or very low (range 0-2.5 nmol/hr/10(6) cells). The absence of activity of 5'-phosphodiesterase was inversely related to the expression of the immune associated antigen (Ia), whereas the absence of Ia correlated with the presence of high activity (range 3.8-16.8 nmol/hr/10(6) cells). A positive low level of terminal deoxynucleotidyl transferase activity was observed in approx 20% of patients but with an activity range (0-30 picomole/min/mg protein) and distribution similar for both subgroups of acute myeloid leukemia. (14 Refs)

51. MONOCLONAL ANTIBODIES TO HUMAN MELANOMA: IDENTIFICATION OF TWO UNIQUE PROTEINS ON THE TUMOR CELL MEMBRANE (MEETING ABSTRACT).

Saxton RE, Mann BD, Irie RF, Morton DL, Burk MW
Sepulveda Veterans Administration Medical Center, Sepulveda, CA, 91343
Proc Am Assoc Cancer Res; 23:1040 1982

Two new monoclonal antibodies produced by hybridomas were identified that define membrane antigens expressed on melanomas but not on autologous or allogeneic B lymphoid cell lines. By indirect protein A assays, monoclonal antibody 436G10 (isotype IgG1) bound to 13/17 melanomas, 3/14 carcinomas and sarcomas, 2/7 gliomas and neuroblastomas, 2/2 fetal cell lines, 0/7 leukemias, and 0/13 lymphoid cell lines. The 436G10 antibody immunoprecipitated a 120 kD protein from 125I-lactoperoxidase-labeled melanoma cells. Monoclonal antibody 705F6 (an IgG2b immunoglobulin) bound to 17/17 melanoma lines, 12/14 carcinoma and sarcoma lines, 7/7 gliomas and neuroblastomas, 3/7 leukemias, 2/2 fetal cell lines, and 0/13 B cell lines. The 705F6 antibody was shown by gel electrophoresis to immunoprecipitate a 95-100 kD protein from labeled melanoma cells. This protein was clearly unrelated to 95-100 kD glycoproteins identified previously on melanomas with monoclonal antibodies by Woodbury et al and Dippold et al. First, the binding patterns of their monoclonal antibodies to our panel of tumor cell lines was different from that of 705F6. Second, 125I-labeled 705F6 binding to melanoma cells was not blocked by any of these antibodies. Because the 705F6 antigen is present on many histologic types of human tumor cells, it will be important to radiolabel this purified antigen and define the levels of this novel protein by radioimmunoassay in sera and tissues of cancer patients. (1 Ref)

52. STUDY OF HUMAN MELANOMA CELL SURFACE ANTIGENS USING MONOCLONAL ANTIBODIES.

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University of Washington, Seattle, WA
Diss Abstr Int [B]; 42(12,pt1):4742-B 1982

BALB/c mice were immunized with cultivated cells from one of two melanomas, M1804 or SK-MEL 28. Spleen cells from hyperimmunized mice were fused with mouse NS-1 myeloma cells. Hybrid cells were grown in HAT (hypoxanthine, aminopterin, thymidine) selective medium and screened for production of antibodies that bound to melanoma cells but not to autologous skin fibroblasts. Five hybrids were selected for extensive studies. Three hybridomas, 3.1, 3.2, and 3.3 form monoclonal antibodies to the same antigenic determinant, called 3.1 which is strongly expressed by the immunizing melanoma, M1804. Binding assays performed with a large panel of cultured human cells have demonstrated that antigen 3.1 is expressed strongly by a small subgroup of cultured melanomas, less strongly by another group of melanomas or not at all by a third group of melanomas. It is not detected on cultures from most non-melanoma tumors, normal skin fibroblast lines, or on B- or T-cells lines. Hybridomas 4.1 and 4.2 were generated against melanoma line SK-MEL 28. Hybridoma 4.1 produces an IgG antibody which identifies a cell surface protein with a molecular weight of 97,000. This protein, designated p97, is strongly expressed by both cultured cells and biopsy material from melanomas and certain other tumors. Cells from various normal tissues express only low amounts of p97. Hybridoma 4.2 secretes an IgM antibody which recognizes an antigen shard by about 95% of melanoma lines and 25% of cultured non-melanoma tumors tested. (no Refs)

53. STRUCTURAL CHARACTERIZATION OF THE HUMAN T CELL SURFACE ANTIGEN (P67) ISOLATED FROM NORMAL AND NEOPLASTIC LYMPHOCYTES.

Fox RI, Harlow D, Royston I, Elder J
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J Immunol; 129(1):401-405 1982

A 67,000-dalton glycoprotein (p67) on normal and neoplastic T cells is defined by reactivity with a monoclonal antibody (antibody SC1) derived against human T chronic lymphocytic leukemia (T-CLL) cells. This antigen is present on greater than 90% of normal thymocytes, normal peripheral blood (PBL) T cells, T acute lymphoblastic leukemia (T-ALL), T-CLL, and B cell chronic lymphocytic leukemia (B-CLL). It is absent from normal B cells, B cell lines, myeloid cells, and stem cell precursors. By using immunoprecipitation of 125I-labeled glycoprotiens and chymotrypsin digestion, the peptide maps of p67 from normal and leukemic lymphocytes revealed that the antigen is highly related among these cell sources, but at least two major forms of the molecule could be identified. One form was prevalent on the T-ALL-derived cell line 8402 and a second form on cell line HPB-T ALL. Peptide maps of PBL from a normal patient, from a T-ALL patient, and from a B-CLL patient were similar to HPB-T ALL cells. In addition to the two major forms of p67, subtle shifts in certain peptides were noted in lymphocytes from each source. The peptide fingerprints of p67 were similar to those obtained from murine Lyt-1 antigen, suggesting possible homology, and were distinctly different from those of murine gp70. Sequential immunoprecipitation experiments verified that the p67 antigen is carried on the same molecule recognized by other independently derived monoclonal antibodies including SC1, L1F12, T101, and OKT1. Cross-blocking experiments indicated that preincubation with antibody L17F12 or T101, but not OKT1, could block subsequent binding of biotin-conjugated antibody SC1. Thus, four of five monoclonal antibodies derived by separate immunizations recognize identical or closely located antigenic sites on p67. (Author abstract) (28 Refs)

54. A HUMAN THY-1-LIKE ANTIGEN EXPRESSED ON CULTURED LEUKEMIC T-CELLS.

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Immunogenetics; 15(6):551-563 1982

A human cell membrane antigen that is highly T-cell specific among a number of leukocyte cell lines was isolated from cells of a human T-cell leukemia cell line, SKW-3. In addition to the high specificity to T-cell-type cell lines, the isolated antigen showed the following characteristics: (1) It is an acidic glycoprotein of approximately 30,000 daltons that has a charge heterogeneity and probably a disulfide bond(s); (2) Its antigenicity is stable when treated with heat, acid, and various protein denaturants; (3) It is widely distributed in lymphoid and nonlymphoid tissues but is most predominant in brain. These features are similar, if not identical, to those reported for the Thy-1 antigen of mouse or rat and have raised the possibility that this cell membrane antigen may correspond to human 'lymphocyte' Thy-1 antigen, the counterpart of human 'brain' Thy-1 antigen. (Author abstract) (28 Refs)

55. MOLECULAR WEIGHT DETERMINATION OF TWO GENETICALLY LINKED CELL SURFACE MURINE ANTIGENS: THB AND LY-6.

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Dept. Immunology, London Hosp. Medical Coll., Turner St., London E1. 2AD, England

Immunogenetics; 15(6):591-599 1982

Various murine tumor lines were screened by FACS analysis for the surface antigens ThB and Ly-6.2. Positive cell lines were used for immunoprecipitation studies. A monoclonal ThB-specific antibody immunoprecipitated a unique acidic protein of approx 16,000 daltons from several positive tumors and from concanavalin A (Con-A) and LPS activated splenic lymphocytes. Monoclonal Ly-6.2-specific antibody was used to immunoprecipitate a 33,500 dalton protein that was shown to exist in four similarly sized forms with different basic charges. In the course of these studies, the apparent molecular weight of the surface antigen T30, immunoprecipitated with a monoclonal T30-specific antibody from the cell line EL4, was found to be approx 25,000 daltons. (Author abstract) (15 Refs)

56. CHARACTERIZATION OF AN AMINO ACID FUCOSIDE OF NORMAL AND SV40-TRANSFORMED HUMAN EMBRYONIC LUNG CELLS.

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Cancer Res; 42(8):3022-3027 1982

The incorporation of radioisotopically labeled fucose into a prominent fucosylated component, ie, fucose-labeled amino acid fucoside 4c (FL4c), of human embryonic lung cells is markedly decreased in cell lines derived from human tumors. In the current study, we have extended the above observations by examining more closely related normal and transformed human cells, eg, W138 cells and SV40-transformed W138 cells. We have found that the level of radioisotopically labeled fucose incorporated into FL4c of the SV40-transformed human embryonic lung cells is dramatically reduced as compared to their normal counterpart cells. Additionally, we have chemically characterized FL4c. FL4c was purified from monkey tissues using a combination of gel filtration chromatography, ion-exchange chromatography, and preparative thin-layer chromatography. Analysis of this material was accomplished by amino acid analyzer and by gas-liquid chromatography; fucose, glucosamine, and aspartic acid in molar ratios of approximately 1.0:1.0:1.0 were observed. Furthermore, alpha-L-fucosidase treatment of [3H]fucose-labeled FL4c revealed that the fucose was alpha-linked and in a terminal position. Similar treatment of [3H]glucosamine-labeled FL4c yielded a component that cochromatographed with N-acetylglucosaminylasparagine. The combined results of the structural studies are consistent with the structure of FL4c being alpha-fucosyl-N-acetylglucosaminylasparagine. (Author abstract) (25 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

57. VITAMIN A DEFICIENCY AND ITS EFFECT ON UROMUCOID CONTENT AND OTHER PARAMETERS RELEVANT TO URINARY CALCULUS DEVELOPMENT IN THE RAT (MEETING ABSTRACT).

Bichler KH, Kirchner C, Strohmaier W, Weiser H, Moormann PS, Korn S, Nedle J
Urologische Abteilung der Universitat Tubingen, Tubingen, W. Germany

6. Symposium fur Experimentelle Urologie, April 15-17, 1982, Bonn, W. Germany. German Association for Urology 155 pp., 1982.

The relationship between vitamin A deficiency and urolithiasis has been described many times without the pathophysiology being understood. Since the influence of vitamin A on glycoprotein synthesis is known the question arises as to whether the mucoproteins of the urinary tract are also controlled by vitamin A. Methods employed included urinalysis: pH, calcium, magnesium, phosphorus, uric acid, citrate, AMPS, total protein, albumin, uromucoid, beta2-microglobulin and sediment; plasma and liver vitamin A concentrations; renal histology and immunofluorescence; bladder histology and calculus analysis. The results show that in vitamin A deficiency calcium elimination in the urine is increased, urinary phosphorus, AMPS and uromucoid are reduced. Vitamin A deficiency also results in a decrease in glycoproteins synthesis in the urinary tract. These are believed to serve a protective function for tubule cells so with insufficient production tubule damage occurs and results in a fall in cell resorption and secretion capacity. The formation of micro- and macrocalculi can develop as a result of the subsequent hypercalciuria and dystrophic calcification. (no Refs) (In GER)

58. HUMAN SQUAMOUS CELL CARCINOMA IN CULTURE: DEFECTIVE TERMINAL DIFFERENTIATION AND INAPPROPRIATE SYNTHESIS OF A SMALL KERATIN (MEETING ABSTRACT).

Rheinwald JG
Sidney Farber Cancer Institute, Boston, MA, 02115

In Vitro; 18(3,part2):319 1982

We have been studying the phenotypes expressed in culture by squamous cell carcinomas (SCCs), using a 3T3 feeder layer system developed for the culture of normal keratinocytes. We have cultured more than 20 SCCs to date; most were from lesions of the oral cavity, which yield cultivatable tumor cells from a majority of biopsies. SCC cells grow with a distinctive, unstratified morphology. They are aneuploid, grow as permanent lines, and form SCCs in athymic mice. The different SCC lines vary greatly in their degree of dependence upon fibroblast feeder support and anchorage. All SCCs exhibits a substantial defect in terminal differentiation, manifest as a resistance to culture conditions that trigger normal karatinocytes to lose division potential and proceed to cornified envelope formation. We have found that SCC cells synthesize only about 1% the normal amount of involucrin, the major envelope subunit protein. The pattern of keratin gene expression is also abnormal in many SCCs compared with their tissue of origin. Of special interest is a small (40 kd) keratin made by some SCCs. An antiserum raised against this protein crossreacts with no other keratin. We and others have recently found that the 40 kd keratin is a normal component of a subset of epithelial cell types, most of which form epithelia other than stratified squamous. (no Refs)

59. ASYNCHRONOUS EXPRESSION OF IMMUNOGLOBULIN GENES IN A HUMAN B CELL LYMPHOMA (MEETING ABSTRACT).

Fan K, Hough AJ
Department of Pathology, Veterans Administration Medical Center, Little Rock, AR

71st Annual Meeting of the International Academy of Pathology (United States-Canadian Division), March 1-5, 1982, Boston, Massachusetts. International Academy of Pathology 95 pp., 1982.

A cell line was established from a well-differentiated human B cell lymphoma. The cloned and suspension-cultured cells synthesized and secreted large amounts (6 per cent of the cytoplasmic protein) of K-light chain without evidence of heavy chain secretion. By immunofluorescence reaction, almost all of the cells showed strongly detectable introcytoplasmic and cell surface K-light chain (cIg+, sIg+), whereas only 5 per cent of the cells showed weakly detectable cytoplasmic mu-heavy chain. After exposure to mitogens, the mu-chain became detectable cytoplasmically in 80 per cent of the cells, whereas the K-chain expression remained unchanged. Furthermore, in a wheat germ cell-free protein synthesis system, the mRNAs isolated from microsomes of the mitogen-unexposed lymphoma cells were able to direct equimolar synthesis of both K- and micro-chain. These findings suggest that expression of light and heavy chain immunoglobulin genes in human B cell may be under separate regulatory mechanisms and that colored B cells can synthesize immunoglobulin subunits asynchronously. In this cell line, it also appears that translational control may play a role in heavy chain gene expression, which is also modifiable by exposure to B cell mitogens such as dextran sulfate or bacterial lipopolysaccharide. (no Refs)

60. THE ANALYSIS OF INTERNALLY RADIOLABELLED MEMBRANE AND SUPERNATANT PROTEINS PRODUCED BY T LYMPHOBLASTOID CELL LINES.

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Diss Abstr Int [B]; 42(12,pt1):4738-B 1982

Proteins from the membranes and culture media of nine, human T lymphoblastoid cell lines and of the myeloid line K 562 were analyzed after [35S] methionine metabolic labelling by electrophoresis in SDS (sodium dodecyl sulfate) polyacrylamide gradient gels. A monotonous consistency was present among these cell lines in their synthesis of membrane proteins. In contrast, the synthesis of culture supernatant proteins was found to differ substantially among the cell lines. Proteins of 35,000 and 36,000 daltons were most prominently synthesized by 1301. Proteins of 50,000 daltons were abundantly synthesized by SKW3 and 8402. Proteins of 65,000 daltons were best synthesized by Jurkat, 1301 and CEM. Synthesis of a CEM supernatant protein of 35,000 daltons (p35), which is scarcely expressed on CEM membranes, increased 16-fold when cells were grown in crowded cultures. Crowded cultures of 1301 but not KE 37 exhibited increased synthesis of p35, while Raji, the only B cell line tested, did not show any synthesis of p35. The view was proposed that cultured T lymphoblastoid malignancies do not represent static vignettes of stages of differentiation of normal T lymphocytes. (no Refs)

61. INHIBITION OF CELLULAR IMMUNITY IN SMALL CELL BRONCHOGENIC CARCINOMA OF THE LUNG: CHARACTERIZATION OF LOW-MOLECULAR SERUM FACTORS WHICH INHIBIT PROTEIN SYNTHESIS IN NORMAL LYMPHOCYTES (MEETING ABSTRACT).

Manke HG, Drings P, Lenhard V, Dirks HP, Toomes H
Onkologisches Zentrum Heidelberg-Mannheim, Mannheim, W. Germany

Onkologie; 4(5):266 1981

The synthesis of RBC-receptor proteins, a differentiation marker of human peripheral T-lymphocytes, is inhibited in patients with small cell bronchogenic carcinoma. This glycoprotein, which has been briefly characterized by Owen and Fanger as well as by Gurtler persists in the membrane for different periods of time depending on the T-cell population. When protein synthesis, nucleocytoplasmic mRNA transfer and mRNA synthesis are inhibited, normal T-lymphocytes are become depleted of RBC-receptor protein with characteristic temporal kinetics. In small cell bronchogenic carcinoma there is a very profound inhibition of the cellular immune system. In a study of 32 patients with small cell bronchogenic carcinoma the defect in cellular immunity correlated with a decrease in RBC-rosette forming cells and patient lymphocyte proliferation in response to mitogenic stimulation. Serum and serum fractions from patients with low RBC-rosette formation inhibited the expression of RBC-receptor protein by normal human peripheral lymphocytes within 60 min when incubated at 37C. This inhibition coincided with a reduction in protein synthesis (H3-amino acid uptake). The inhibitory serum fraction was further purified by means of high pressure liquid chromatography; the inhibitory trypsin-sensitive fraction lies in the 20,000 dalton region. The present studies refer to an inhibition of protein synthesis possibly on account of induction of a cyclic AMP-dependent protein kinase. (no Refs) (In GER)

62. HYPERTHERMIA AND THE HEAT-SHOCK PROTEINS OF HELA CELLS.

Burdon RH, Slater A, McMahon M, Cato AC
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Br J Cancer; 45(6):953-963 1982

When HeLa cells are subject to hyperthermia, the synthesis of specific heat-shock proteins (HSP) is induced under a variety of thermal conditions. HSP synthesis does not occur at temperatures above 43 C but requires return to a culture temperature of 37 C. Maximal induction appears to be achieved if a brief hyperthermia treatment (10 min, 45-46 C) is followed by 2 h 'development' at 37 C. The induction process requires transcription but not DNA replication, and general cell metabolism is probably also required, as induction does not occur if the heat-treated cells are returned to 4 C (rather than 37 C) for development. A small proportion of the HSPs of 72-74 Kd are found in nuclei, but do not appear to bind to DNA. The bulk of these proteins, as well as those at 100 Kd, are cytoplasmic, but none are preferentially associated with mitochondria. Increased synthesis of the 100Kd and 72-74Kd HSPs was also triggered by pretreatment of the cells with 5 x 10-5M sodium arsenite. (Author abstract) (34 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

63. INDUCTION OF BENZO[A]PYRENE HYDROXYLASE IN SKIN AND LIVER BY CUTANEOUS APPLICATION OF JUTE BATCHING OIL.

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Industrial Toxicology Res. Centre, Mahatma Gandhi Marg, Lucknow 226001, India

Toxicology; 23(4):347-352 1982

The ability of jute batching oil (JBO; two varieties) to pass through the skin, either metabolized or not; the biochemical changes in skin and liver following cutaneous exposure to JBO; and the duration of the effects in both tissues after a single painting were investigated. Female albino mice of ITRC strain were used in skin painting experiments and as sources of skin and liver tissue microsomal preparations. Cutaneous application of JBO for 8 days resulted in increased liver wt (35%) and hepatic microsomal protein content (30%). Benzo[a]pyrene hydroxylase (BAPH) activity in treated liver and skin was enhanced 2-and 10-fold, respectively, but hepatic aminopyrene demethylase activity was unaltered. Catalytically and spectrally distinct microsomal cytochrome P-448 levels were increased in hepatic and skin microsomes from animals treated with JBO. A single skin painting increased BAPH activity in skin and liver to its maximum after 1 and 2 days, respectively. The higher enzyme activity persisted up to 6 days in skin and up to 14 days in liver, thereafter declining more rapidly in skin than liver. JBO, by virtue of its ability to enhance BAPH activity locally in the skin at the site of contact or distantly in liver after passage through the blood seems to inadvertently stimulate the metabolism of its own components. This behavior may contribute electrophilic metabolites to the epidermis, subsequently leading to neoplastic transformation of the cells. (16 Refs)

64. ELEVATION OF SERUM ALBUMIN CONCENTRATION IN ANALBUMINEMIC RATS BY ADMINISTRATION OF 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE.

Makino R, Esumi H, Sato S, Takahashi Y, Nagase S, Sugimura T
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Biochem Biophys Res Commun; 106(3):863-870 1982

Analbuminemic rats established from a stock of Sprague-Dawley rats were investigated for the presence of rat serum albumin and the effects of 3'-methyl-4-dimethylaminoazobenzene (DAB) on albumin content in these rats was investigated. In 4-wk-old rat sera a single radial immunodiffusion method detected a small but distinct precipitin ring that was indistinguishable from rat serum albumin (RSA) by immunoelectrophoresis. The protein had the same mol wt of 65,000 as RSA and reacted with anti-RSA IgG. Peptide mapping demonstrated that the protein was albumin-like and further investigation demonstrated that it originated from the analbuminemic rats. Treatment of the rats with DAB for 7 wk increased the serum albumin concentration to a max after 15 wk (32 ug/ml, approx 2x that of untreated rats). One rat autopsied after 18 wk of DAB treatment had a hepatic tumor. It is suggested that somatic reverse mutation of analbuminemic rat hepatocytes caused the increase of serum albumin observed after DAB treatment. (18 Refs)

65. RETINOIDS INCREASE TRANSGLUTAMINASE ACTIVITY AND INHIBIT ORNITHINE DECARBOXYLASE ACTIVITY IN CHINESE HAMSTER OVARY CELLS AND IN MELANOMA CELLS STIMULATED TO DIFFERENTIATE.

Scott KF, Meyskens FL, Russell DH
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Proc Natl Acad Sci USA; 79(13):4093-4097 1982

Transglutaminase (TGase; R-glutaminyl-peptide:amine gamma-glutamyltransferase, EC 2.3.2.13) and ornithine decarboxylase (ODCase; L-ornithine carboxy-lyase, EC 4.1.1.17) activities were measured after the addition of retinoid analogs to Chinese hamster ovary (CHO) cells released from quiescence and Cloudman S91 (CCL 53.1) mouse melanoma cells stimulated to differentiate with alpha-melanocyte-stimulating hormone (MSH, melanotropin). In both cell culture lines, we detected a biphasic increase in TGase activity and a single peak of ODCase activity within 7 hr after release or stimulation. Retinoid analogs altered the expression of the initial TGase peak in both CHO and melanoma cells. Retinol increased the activity of TGase 1 hr after release in CHO cells, and the activity remained elevated until hr 4. A broad peak of TGase activity also occurred after the addition of alpha-difluoromethylornithne, an irreversible inhibitor of ODC-ase, and after addition of alpha-difluoromethylornithine plus retinol. In mouse melanoma cells, retinoic acid plus MSH markedly enhanced the activity of the initial TGase peak compared to MSH alone. Retinoic acid alone also increased TGase activity biphasically in these cells without the addition of MSH. These studies suggest that retinoid effects that increase TGase activity may alter the ODCase expression in proliferation and differentiation. (Author abstract) (36 Refs)

66. DIRECT ACTIVATION OF CALCIUM-ACTIVATED, PHOSPHOLIPID-DEPENDENT PROTEIN KINASE BY TUMOR-PROMOTING PHORBOL ESTERS.

Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y
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J Biol Chem; 257(13):7847-7851 1982

Tumor-promoting phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) directly activate in vitro Ca++-activated, phospholipid-dependent protein kinase (protein kinase C), which normally requires unsaturated diacylglycerol. Kinetic analysis indicates that TPA can substitute for diacylglycerol and greatly increases the affinity of the enzyme for Ca++ as well as for phospholipid. Under physiological conditions, the activation of this enzyme appears to be linked to the receptor-mediated phosphatidylinositol breakdown which may be provoked by a wide variety of extracellular messengers, eventually leading to the activation of specific cellular functions or proliferation. Using human platelets as a model system, TPA is shown to enhance the protein kinase C-specific phosphorylation associated with the release reaction in the total absence of phosphatidylinositol breakdown. Various phorbol derivatives which have been shown to be active in tumor promotion are also capable of activating this protein kinase in vitro systems. (Author abstract) (39 Refs)

67. ALTERATIONS IN CELLULAR MORPHOLOGY, PROLIFERATIVE RATE AND PEPTIDE COMPOSITION ACCOMPANY DIMETHYLSULFOXIDE-ENHANCED LIVER PROTEIN SYNTHESIS BY HEPATOMA CELLS.

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Cell Mol Biol; 28(3):299-305 1982

The effect of the polar solvent dimethylsulfoxide on the growth, morphology and protein composition of malignant hepatocytes was studied in an established line of mouse hepatoma cells. Dimethylsulfoxide decreased final hepatocyte population density, altered culture morphology from a random monolayer-type of growth to one in which hepatocytes were organized into discrete colonies and produced a dose-dependent enhancement in expression of the liver-specific proteins albumin and transferrin. In addition, the concentration of two peptides of 96,000 and 110,000 daltons in mass was significantly enhanced in dimethylsulfoxide-stimulated hepatoma cells. The levels of these peptides in cellular lysates was inversely related to the proliferative status of the culture, suggesting that they may serve as staging markers of hepatocyte growth states. (Author abstract) (19 Refs)

68. EFFECTS OF HEXAMETHYLENE BISACETAMIDE (HMBA), SODIUM BUTYRATE (BA) AND DIMETHYL SULFOXIDE (DMSO) ON ALPHA-FETOPROTEIN (AFP), ALBUMIN AND TRANSFERRIN PRODUCTION BY A HEPATOMA CELL LINE IN SERUM-FREE MEDIUM (MEETING ABSTRACT).

Hughes EH, Kim N
National Cancer Institute, Bethesda, MD, 20205
Proc Am Assoc Cancer Res; 23:145 1982

Low molecular weight compounds such as DMSO and BA are known to modulate the production of AFP, albumin and transferrin in hepatoma cell lines. In order to further study these effects of DMSO, BA as well as HMBA, growth of rat hepatoma cell line McA-RH 7777 (7777) was obtained on plates precoated with human fibronectin in a serum-free medium. Concentrations of AFP, albumin and transferrin were determined by radioimmunoassay. The medium consisted of 1:1 mixures of Ham's F-12 and Dulbecco's modified Eagles supplemented with insulin, iron saturated transferrin and selenium and 15 mM Hepes. The production of AFP, and transferrin was 200-350 and 15-30 ng/ 10(5) cells/24 hr, respectively. Albumin was nondetectable. Treatment with BA induced a dose-dependent increase in synthesis of AFP and transferrin whereas treatment with HMBA and DMSO caused a dose-dependent decrease in the synthesis of both AFP and transferrin. No induction of albumin synthesis was observed. Similar effects of all three agents on 7777 are observed after treatment with DMSO and BA in serum supplemented medium, except that albumin synthesis was induced 6-fold with BA and HMBA induced AFP synthesis 2-fold. The capacity of BA (4 mM) to induce albumin secretion and of HMBA (4 mM) to induce AFP secretion under serum containing growth conditions but not in defined medium may reflect an absence of permissive factor(s) in the defined medium. (1 Ref)

69. EFFECTS OF HYPERTHERMIA ON CLEARANCE OF ASIALO-FETUIN (AF) BY THE PERFUSED RAT LIVER (MEETING ABSTRACT).

Skibba JL, McKean L, Winkelhake JL
The Medical College of Wisconsin, Milwaukee, WI, 53226
Proc Am Assoc Cancer Res; 23:758 1982

Plasma membrane and lysosomes have been identified as targets for hyperthermic damage. The glycoprotein, AF, was used to assess effects of heat on this protein clearance and degradation by the perfused liver. AF enters hepatocytes by a receptor-mediated endocytosis and becomes localized in the lysosomes. Rat livers were perfused in situ with diluted whole rat blood (1:3). After 30 min, a mixture of 125I-Fetuin (F), and 131I-AF was added. Uptake was measured as disappearance of acid precipitable (AP) material and proteolysis by appearance of acid soluble (AS) activity. The 125I-F serves as an internal control. Results show a similar rate of clearance of AF at 37, 41 and 42 degrees. At 41 and 42 degrees there is a decrease in AS activity in the perfusate. This is accompanied by a greater than 10-fold increase in the ratio of cpm/gm liver to ml perfusate at the end of the 2 hr perfusion. These data show that an increase in temperature to 41 and 42 degrees did not alter hepatic clearance of AF, but did suggest that lysosomal proteolysis was inhibited at 41 and 42 degrees. (no Refs)

70. ESTROGENS STIMULATE CELL PROLIFERATION AND INDUCE SECRETORY PROTEINS IN A HUMAN BREAST CANCER CELL LINE (T47D).

Chalbos D, Vignon F, Keydar I, Rochefort H
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J Clin Endocrinol Metab; 55(2):276-283 1982

The effects of estradiol (E2) on two cloned sublines derived from the T47D human breast cancer cell line have been studied in vitro. Cell proliferation was evaluated by DNA assay, cell counts, and thymidine incorporation. The rate of synthesis of proteins released into the cell culture medium was assayed by [35S]methionine incorporation and polyacrylamide gel electrophoresis, followed by fluorography. In clone 11, which contains estrogen and progesterone receptors, estradiol (1 pM-1 nM) stimulated cell proliferation 2-to 5-fold after a lag period of 6 days. Maximal stimulation was observed with 1% of 3% fetal calf serum and without added insulin. The effect of E2 was biphasic, since the growth rate was stimulated for E2 concentrations less than 10 nM and then progressively inhibited for higher concentrations. Dexamethasone, dihydrotestosterone, progesterone, and R5020 (at 1 nM or 1 uM) did not modify cell growth. The antiestrogen Tamoxifen (1 uM) inhibited the E2-induced stimulation and decreased the growth of control cells. Estrogen also stimulated 2-to 3-fold the synthesis of approximately 60K mol wt dalton proteins which were released into the medium. This effect was seen as early as 1 day of E2 treatment, and a plateau of stimulation was reached with 0.1 nM E2. By contrast, in clone 8, which contains low concentrations of estrogen and progesterone receptors. E2 had no effect on cell growth and stimulated slightly the synthesis of a 55K protein. These results demonstrate that E2 is able to directly stimulate the proliferation of human epithelial breast cancer cells after having stimulated the synthesis of approximately 60,000 dalton proteins released into the medium. (Author abstract) (30 Refs)

71. INDUCTION OF CYCLIC AMP-DEPENDENT PHOSPHORYLATION IN CYTOSOL OF DIFFERENTIATING MOUSE ERYTHROLEUKEMIA CELL LINE 179.

Emler CA, Fontana JA, Butcher FR
(c/o Butcher) Dept. Biochemistry, West Virginia Univ. Medical Center, Morgantown, WV, 26506
FEBS Lett; 142(2):210-214 1982

The effects of calcium and cyclic AMP (cAMP) on the in vitro phosphorylation of proteins in a soluble fraction from mouse erythroleukemia (MEL) cells at various times during the process of differentiation were studied. A marked decrease in phosphorylation of a 120,000-mol wt protein occurred with the addition of dimethylformamide that was independent of cAMP and calcium. A specific cAMP-dependent protein phosphorylation at mol wt 70,000 occurred within 24 hr of addition of inducer, regardless of which inducer was used. Early cAMP-dependent protein phosphorylation events are crucial in the differentiation process. (18 Refs)

72. LUTROPIN INCREASES PHOSPHORYLATION OF A 33,000-DALTON RIBOSOMAL PROTEIN IN RAT TUMOUR LEYDIG CELLS.

Bakker GH, Hoogerbrugge JW, Rommerts FF, Van der Molen HJ
Dept. Biochemistry, Medical Faculty, Erasmus Univ., Rotterdam, The Netherlands
Biochem J; 204(3):809-815 1982

Localization of lutropin-dependent phosphoproteins demonstrated in tumor Leydig cells which might be involved in regulation of steroidogenesis was further investigated. Results indicated that a lutropin-dependent phosphoprotein of 33,000 dalton was present in the 40S ribosomal subunit isolated from tumor Leydig cells. Phosphorylation of the protein was stimulated by cycloheximide and puromycin, under conditions in which basal and lutropin-dependent pregnenolone productions were inhibited. The decrease with time in pregnenolone production under basal and stimulated conditions was approx the same, which suggests the possible involvement of proteins with a short half-life (approx 6 min). (20 Refs)

73. THE TURNOVER OF HEPATOMA CELL GAMMA-GLUTAMYL TRANSFERASE.

Ding JL, Smith GD, Peters TJ
(c/o Smith) Div. Clinical Cell Biology, MRC Clinical Res. Centre, Watford Road, Harrow, Middlesex HA1 3UJ, England
FEBS Lett; 142(2):207-209 1982

The rate of breakdown of gamma-glutamyl transferase (GGT) in cultured hepatoma cells was followed during the inhibition of protein synthesis by cycloheximide and the rate of synthesis of GGT was monitored after covalent inactivation of the pre-existing levels of GGT. Hepatoma GGT had a half-life of 24 hr, compared with 3 hr for that of the normal liver GGT, explaining the increase in GGT activity of hepatoma cells. (20 Refs)

DIETARY AND NUTRITIONAL EFFECTS

74. METHIONINE REGULATION OF N-5-METHYLTETRAHYDROFOLATE:HOMOCYSTEINE METHYLTRANSFERASE AND ITS INFLUENCE ON THE GROWTH AND PROTEIN SYNTHESIS IN NORMAL, NEOPLASTIC, AND TRANSFORMED CELLS IN CULTURE.

Tautt JW, Anuszewska EL, Koziorowska JH
Inst. Drug Res. Control, 00-725 Warsaw Chemska 30/34, Poland
JNCI; 69(1):9-14 1982

Normal human embryonic fibroblasts (CL-58) and normal embryonic rat fibroblasts (REF) revealed equal growth in media containing 0.2 mM DL-homocysteine thiolactone (HOM) or in methionine (METH) enriched with 1.5 uM cyanocobalamin and 0.1 mM folic acid. Whereas L 5178Y lymphoblasts and transformed rat fibroblasts (REF-S) showed more or less acute need for exogenous METH. In normal cells general protein synthesis remained unimpaired during 24 hr after substitution of METH by HOM. In L 5178Y and REF-S cells, however, protein synthesis was significantly inhibited. Measurements of N-5-methyltetrahydrofolate:homocysteine methyltransferase revealed a two- to three fold increase in enzyme activity in normal cells grown on HOM and in L 5178Y and REF-S cells grown on HOM with added METH. HeLa cells showed variability in growth pattern, in general protein synthesis response, and in inducibility of methyltransferase upon substitution of METH by HOM. (Author abstract) (23 Refs)

POLYAMINES - SYNTHESIS AND METABOLISM

75. EFFECT OF AFLATOXIN B1 ON CROTON OIL INDUCED ALTERATIONS IN HEPATIC POLYAMINES.

Chaturvedi A, Raj HG, Venkitasubramanian TA
(c/o Raj) Dept. Biochemistry, Vallabhbhai Patel Chest Inst., Delhi Univ., Delhi 110 007, India
IRCS Med Sci [Cancer]; 9(8):730 1981

Administration of croton oil (CO: 10 mg/kg) to Swiss mice significantly elevated the levels of hepatic polyamines. Aflatoxin B1 (AFB: 12 mg/kg) or actinomycin D (ACD: 0.7 mg/kg) further enhanced mouse liver putrescine content. Administration of AFB and ACT simultaneously to CO-treated mice resulted in significant reductions in the concentration of liver putrescine. AFB alone and in conjunction with CO consistently elevated liver polyamine levels, indicating that AFB can to some extent act as a promoter. (7 Refs)

76. INVOLVEMENT OF POLYAMINES IN DIFFERENTIATION OF FRIEND ERYTHROLEUKEMIA CELLS: STUDIES WITH DIACETYL-14C PUTRESCINE (DDAB) (MEETING ABSTRACT).

Gazitt Y, Bondy PK, Canellakis ZN
Department of Immunology, Hebrew University, Hadassah Medical School, Jerusalem, Israel
Proc Am Assoc Cancer Res; 23:899 1982

Polyamines and ornithine decarboxylase play a role in induction of differentation in Friend erythroleukemia (FL) cells. We now report the uptake and metabolism of DDAB in FL cells induced to differentiate by 10 mM DDAB (1 uCi/mole). The results indicate a progressive increase in uptake of inducer with 90% benzidine positive cells on day 3. By 24 hours up to 35% of cytosolic DDAB was metabolized and appeared as N-acetyl-putrescine (2%), putrescine (25%), N-acetyl spermidine (0.3%), spermidine (5%), and spermine (1.5%). Cytosolic concentrations of the major polyamines, putrescine, spermidine, and spermine were increased 4.2, 1.2, and 1.06 fold respectively. When dexamethasone (Dex, 2 uM) was added to cultures induced by DDAB it caused decreases in cytosolic concentration of DDAB (50%) by 24 hours, as well as changes in the concentration of putrescine (from 400 uM to 280 uM), spermidine (360 uM to 140 uM), and spermine (250 uM to 70 uM). The ratios of radioactive/endogenous polyamines in the presence of Dex were modified as follows: putrescine (from 3.1 to 0.6), spermidine (from 0.16 to 0.6) and spermine (from 0.16 to 0.6) and spermine (0.06 to 0.1); there was also a complete disappearance of monoacetyl putrescine, and N-acetyl spermidine. By contrast, both nuclear and membrane associated polyamines showed increases of DDAB metabolites in response to Dex. (1 Ref)

77. METABOLISM OF AMINO ACIDS AND POLYAMINES IN MALIGNANT TUMORS.

Berezov TT
P. Lumumba Univ. People's Friendship, Moscow, USSR
Lab Delo; (5):292-294 1982

Literature data on amino acid and polyamine metabolism in malignant tumors are briefly reviewed. Analyses of various human and experimental neoplasms showed a progressive loss of activity of key enzymes of nitrogen metabolism (threonine dehydratase, serine dehydratase, histidase, diamine oxidase, and asparagine synthetase). (21 Refs) (In RUS)

OTHER RELATED STUDIES

78. DIFFERENCES IN PATTERNS OF COMPLEMENTATION OF THE MORE COMMON GROUPS OF XERODERMA PIGMENTOSUM: POSSIBLE IMPLICATIONS.

Giannelli F, Pawsey SA, Avery JA
Paediatric Res. Unit, Guy's Hops., London SE1 9RT, England
Cell; 29(2):451-458 1982

Xeroderma pigmentosum of Groups A, C and D shows complementation differing in kinetics, dependence on the dose of wild-type alleles and dependence on protein synthesis. Such differences suggest that XP-A, -C and -D carry mutations at different loci. The product of the first of these loci (factor A) is present in significant excess in normal fibroblasts, seems to turn over rapidly and may be a dimer or higher polymer. The products of the other two loci (factors C and D) do not seem to be present in significant excess in the cytoplasm of normal fibroblasts, but factor C may accumulate abnormally in XP-D. Factors C and D turn over slowly (D more than C) and they do not move freely from the cell nucleus. Factors A and C, at least, seem to act directly and not via gene regulation. (Author abstract) (17 Refs)

79. EFFECT OF GUCAGON ON AMINO ACID TRANSPORT AND CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE PRODUCTION IN RAT HEPATOMA CELL LINE MCA-RH 8994 IN CULTURE.

Kelley DS
Dept. Biochemistry, West Virginia Univ., Sch. Medicine, Morgantown, WV, 26506
Cancer Res; 42(8):3116-3119 1982

The rates of alpha-aminoisobutyrate (AIB) transport and cyclic adenosine 3':5'-monophosphate (cAMP) production in response to glucagon (2 x 10(-7) M) have been determined in monolayer cultures of hepatoma cell line 8994 (refes to hepatoma cell line McA-RH 8994) under three different calcium treatments. The calcium treatments were: (a) control, cultures maintained in calcium-containing medium without prior calcium depletion; (b) calcium depleted, cultures maintained in calcium-free medium after treatment with ionophore A23187 and ethylene glycol bis(beta-aminoethyl ether)-N,N'-tetraacetic acid; (c) calcium repleted, cultures maintained in calcium-containing medium after treatment with A23187 and ethylene glycol bis (beta-aminoethyl ether)-N,N'-tetraacetic acid. In the control cultures, glucagon in the absence of methylisobutylxanthine (MIX) has no effect on the rate of AIB transport. AIB transport is approx doubled when glucagon is added in the presence of MIX. Calcium depletion and calcium repletion enhance the rate of AIB transport by about 50 and 100%, respectively. Glucagon and N6,O2-dibutyryl cyclic adenosine 3';5'-monophosphate (1 x 10(-4) M) further increase the rate of AIB transport by 10% in calcium-depleted cultures and by 50% in calcium-repleted cultures. The stimulation of AIB transport by glucagon as well as by the ionophore can be blocked by the simultaneous addition of cycloheximide (0.1 mM) or actinomycin D (3.2 uM). In the absence of MIX, there is about a two-fold increase in the level of cAMP in response to glucagon. In the presence of MIX, glucagon causes more than 100-fold increase in the level of cAMP. The presence or absence of calcium does not affect the basal or glucagon-stimulated production of cAMP in these cultures. We have reported previously that, in monolayer cultures of rat hepatocytes, calcium repletion enhanced the glucagon stimulation of AIB transport while it markedly inhibited the production of cAMP in response to glucagon. It is suggested that calcium rather than cAMP has a direct effect on the amino acid transport system. (Author abstract) (24 Refs)

80. MODIFICATIONS OF SMALL INTESTINE LYSOSOMAL ENZYMES AFTER IRRADIATION AT DIFFERENT TIMES OF THE DAY.

Becciolini A, Giache V, Lanini A, Cremonini D, Drighi E
Inst. Radiology, Medical Sch., Univ. Florence, I-50134 Florence, Italy
Acta Radiol Oncol Radiat Phys Biol; 21(1):61-66 1982

The modification of lysosomal enzyme activities in animals irradiated with the same sublethal dose at four different times of the day is reported. The results confirmed the absence of circadian fluctuations in all the lysosomal enzymes and in protein content. A difference in behavior between acid beta-galactosidase and beta-glucuronidase on the one hand and between acid phosphatase and cathepsin D on the other was evident in irradiated animals. The results showed that acid beta-galactosidase and beta-glucuronidase increase from the early intervals after irradiation and reach the highest activity between 36 and 48 hr. At these intervals autolysis phenomena, heavy cellular alterations and numerous phlogosis cells are present in the epithelium. Only beta-glucuronidase and acid beta-galactosidase indicate the level of radiation injury. (Author abstract) (19 Refs)

81. CHANGES IN [3H]GALACTOSE UPTAKE IN HUMAN COLONIC MUCOSA WITH CARCINOMA: AN ULTRASTRUCTURAL STUDY.

Dawson PA, Filipe MI
Dept. Histopathology, Westminster Medical Sch., Udall St., London SW1P 2PP, England
Histochem J; 14(3):361-383 1982

Uptake of [3H]galactose was studied by electron microscopical autoradiography both on the basis of isotope uptake over a period of time and with pulse labeling to investigate the changes in the glycoprotein synthesis associated with malignancy in the colonic mucosa. Normal mucosa and mucosa adjacent to tumor ('transitional') from human large bowel specimens resected for carcinoma were compared. The most marked differences were found in the middle and upper crypt; the region of differentiation being the middle crypt in all types of tissue and the upper crypt being a region of extended immaturity in transitional mucosa. (24 Refs)

82. UPTAKE OF [3H]THREONINE IN HUMAN COLONIC MUCOSA ASSOCIATED WITH CARCINOMA: AN AUTORADIOGRAPHIC ANALYSIS AT THE ULTRASTRUCTURAL LEVEL.

Dawson PA, Filipe MI
Dept. Histopathology, Westminster Medical Sch., Udall St., London SW1P 2PP, England
Histochem J; 14(3):385-401 1982

[3H]Threonine uptake was studied by electron microscopical autoradiography using the method of statistical analysis of Blackett and Parry. The results are discussed in the light of [3H]galactose studies. The changes in protein uptake are compared with different isotope incubation periods and with pulse-labeling techniques. Mean reactive values, including the standard errors, for the control and the 'transitional' mucosa with [3H]threonine labeling for the various organelles are presented. Results indicated that the increased uptake of [3H]threonine in transitional mucosa appears to be the result of prolonged protein synthesis associated with an extension of the cellular proliferation zone in the crypt rather than being an effect of increased cell turnover. (14 Refs)

83. NEUROCHEMICAL ACTIONS AND ROLES OF METHIONINE: IN VITRO STUDIES USING THE RAT C6 ASTROCYTOMA CELL AS A MODEL SYSTEM.

Kohl RL
The University of Texas Graduate School of Biomedical Science at, Galveston Galveston, TX
Dis Abstr Int [B]; 42(8):3236-B 1982

C6 cells contain supranormal levels of serine hydroxymethyltransferase (EC 2.1.2.1)- specific activity compared to non-neoplastic cells. They excreted large amounts of glycine in vitro which raised the question of what role the glial cell has in glycine neurotransmission. It was concluded that C6 cells are unsuitable for answering this question because their high serine hydroxymethyltransferase-specific activity may indicate cell transformation. Although enzymatic activity was not respressible by methionine, glycine, serine, adenine, guanine or thymine, other evidence suggested intact allosteric regulation by 5-methyltetrahydrofolate. Most significantly, acceleration of the conversion of serine to glycine followed administration of methionine. This implied the occurrence of one-carbon metabolism activation via mobilization of carbon fragments from serine. Intracellular GABA (gamma-aminobutyric acid) levels increased in C6 cells incubated in 1.2mM methionine. Concomitant alterations in glutamine and glutamate metabolism occurred. The contribution of putrescine to total cellular production of GABA was insignificant. C6 cells possess enzymes for methionine biosynthesis from homocysteine and formation of cysteine. Rapid metabolism of N-methylglycine occurred. Cells demonstrated toxic reactions to 1.2 mM alpha-keto-gamma-methiolbutyrate and methionine sulfoxide but not to 1.2 mM ethionine. It is unknown whether high, unregulated levels of serine hydroxymethyltransferase are present in normal mammalian astrocytes. It also remains to be determined whether these high levels occur after neoplastic transformation. (no Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Alkjaersig N	36
Antony M	63
Anuszewska EL	74
Artlieb U	9
Ashall F	48
Avery JA	78
Bakker GH	72
Bano M	29
Barlow JJ	14
Basu TK	44
Baxter CS	8
Becciolini A	80
Berezov TT	77
Berger SL	24
Bhattacharya M	14
Bichler KH	57
Biswas DR	40
Bondy PK	76
Bramwell ME	48
Briggs RC	6
Brown TJ	41
Buchanan JH	1
Buehler R	17
Burdon RH	62
Burk MW	51
Burtin P	23
Butcher FR	71
Canellakis ZN	76
Cassaro-Strunz	32
Castagna M	66
Cato AC	62
Chakrabarti S	40
Chalbos D	70
Chan AW	33
Chatterjee SK	14
Chaturvedi A	75
Chavanel G	23
Cremonini D	80
Crowne HM	1
Dawson PA	81
Dawson PA	82
De Fatima Bonal	32
DeMartino JL	60
Delikatny J	34
Denk H	9
Deutsch HF	26
Ding JL	73
Dirks HP	61
Drighi E	80
Drings P	61
Durham JB	42
Dvorak AM	37
Dvorak HF	37
Ebbers J	31
Elder J	53
Emler CA	71
Esumi H	64
Fan K	59
Filipe MI	81
Filipe MI	82
Fletcher AP	36
Foidart JM	23
Fontana JA	71
Fox RI	53
Franke WW	9
Fujita T	47
Fujiyama Y	20
Galvan LM	38
Ganzer U	31
Gazitt Y	76
Giache V	80
Giannelli F	78
Giavazzi R	35
Gillner M	46
Ginsberg BH	41
Glaudemans CP	27
Gropp C	22
Gustafsson JA	46
Hachiya T	20
Hanes SD	40
Harlow D	53
Harris H	48
Harris NL	37
Havemann K	22
Herzenberg LA	55
Hess M	17
Higashiyama T	16
Higgins PJ	67
Hnilica LS	6
Hogberg B	46
Hoogerbrugge JW	72
Hosoda S	20
Hough AJ	59
Hughes EH	68
Huhtala ML	21
Ichikawa Y	11
Iqbal MP	15
Irie RF	51
Isersky C	45
Ishizeki J	10
Iwamura N	47
Jabusch JR	26
Johansson G	46
Jones L	44
Kaibuchi K	66
Kajita Y	20
Kamei T	10
Kawashima R	47
Kellen JA	33
Kelley DS	79
Keydar I	70
Kidwell WR	29
Kikkawa U	66
Kim N	68
Kirchner C	57
Klinger MM	56
Ko CY	30
Kohl RL	83
Kohn J	44
Koistinen R	21
Konishi Y	47
Korn S	57
Korotkoruchko V	28
Koziorowska JH	74
Krepler R	9
Kuhnlein U	4
Kumar S	63
Lackinger E	9
Lambert MW	5
Lanini A	80
Ledbetter JA	55
Lenhard V	61
Letnansky K	3
Liotta LA	24
Lo KW	49
Lobanenkov VV	2
Lockwood AH	19
Losa G	50
Luster W	22
Machado-Santell	32
Makino R	64
Malkin A	33
Malkin DG	33
Manjula BN	27
Manke HG	61
Mann BD	51
Martin E	23
Matossian-Roger	55
Matsuyama M	12
McGuire J	5
McKean L	69
McMahon M	62
Medvedev ZA	1
Medvedeva MN	1
Mehrotra NK	63
Metzger H	45
Meyskens FL	65
Mironov NM	2
Mitra R	42
Miyazaki T	20
Mokashi S	34
Moore JJ	43
Moormann PS	57
Mori T	10
Morita T	12
Morton DL	51
Morton PA	56
Mu MC	40
Nagase S	64
Nagata K	11
Nakagawa T	47
Nakamura H	12
Nakamura T	16
Nakata M	47
Nakazato Y	10
Nedle J	57
Nishizuka Y	66
Noteboom WD	42
Ochi Y	20
Orr FW	34
Osawa Y	16
Ossowski L	25
Patskan GJ	8
Pawsey SA	78
Pendergast M	19
Peters TJ	73
Potter M	27
Priest RE	30

Protsenko BA 28
Raj HG 75
Reano A 18
Reich E 25
Rheinwald JG 58
Rivera J 45
Rochefort H 70
Rogers P 55
Rommerts FF 72
Rowlands L 44
Royston I 53
Russell DH 65
Russo RG 24
Sagara J 11
Saji F 54
Sakata T 7
Salomon D 29
Sano K 66
Sato S 64
Saxton RE 51
Schmidt WN 6
Scott KF 65
Sen'ko LN 28
Seppala M 21
Shapot VS 2
Shur BD 39
Siegal GP 24
Skibba JL 69
Slater A 62
Smith GD 73
Smith J 37
Steiner SM 56
Stenman UH 21
Strohmaier W 57
Stryjecka-Zimme 6
Suganuma T 49
Sugimura T 64
Takahashi K 10
Takahashi Y 64
Takai Y 66
Takenaka M 47
Tanabe K 12
Tanigaki N 54
Tautt JW 74
Thivolet J 18
Thorgeirsson UP 24
Tochigi B 16
Tomita M 47
Toomes H 61
Toshima K 13
Triche TJ 45
Trivette DD 19
Tsang SS 4
Tsou KC 49
Tsuboi S 47
Van der Molen H 72
Venkitasubraman 75
Viac J 18
Vignon F 70
Von Wartburg JP 17
Wallace DM 24
Weiser H 57
Whitsett JA 43
Winkelhake JL 69
Witzleben CL 49
Workman L 43
Wun TC 25
Yamaguchi H 10
Yamamoto T 16
Yarborough C 16
Yeh MY 52
Yoshida S 12
Yoshimura M 20
Zwiebel J 29

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In this issue:

- NUCLEOPROTEINS 1-4
- CYTOPLASMIC AND CONTRACTILE PROTEINS 5-14
- PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS 15-42
- BINDING/ RECEPTOR PROTEINS 43-54
- MEMBRANE/ SURFACE PROTEINS 55-86
- STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS 87-100
- EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM 101-113
- DIETARY AND NUTRITIONAL EFFECTS... 114-115
- OTHER RELATED STUDIES 116-131

NUCLEOPROTEINS

1. NATURE OF THE CONSTITUTIVE AND INDUCED MAMMALIAN O6-METHYLGUANINE DNA REPAIR ENZYME.

Craddock VM, Henderson AR, Gash S
MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey, England
Biochem Biophys Res Commun; 107(2):546-553 1982

Previous studies indicate that removal of O6-methylguanine (O6-MG) from DNA occurs by transfer of the methyl group from the O6-position of guanine to a cysteine residue in protein, forming acid-stable S-methylcysteine (S-MC). However, there is evidence that the reaction product is acid labile. This inconsistency was investigated. The amount of O6-MG removed from DNA by extracts or rat liver and Raji cells in vitro was compared with the amount of S-MC formed. The O6-MG DNA repair enzyme was extracted from rat liver as previously described. The DNA used as substrate was methylated with [3H-methyl]nitroso-methylurea. The enzyme concentration curve showed that the amount of S-MC formed was proportional to the amount of enzyme added. The nature of

the increased ability of liver of diethylnitrosamine (DEN)-treated rats to remove O6-MG was also investigated by study of enzyme activity of liver extracts. DEN treatment increased repair enzyme activity approx 3x. The data supports the view that removal of O6-MG from DNA occurs by transmethylation, the methyl group giving rise to acid-stable S-MC residues in protein. The induced enzyme, like the constitutive enzyme, appears to be a transmethylase. (26 Refs)

2. EVIDENCE FOR TWO CLASSES OF CHROMATIN-ASSOCIATED EPSTEIN-BARR VIRUS-DETERMINED NUCLEAR ANTIGEN.

Spelsberg TC, Sculley TB, Pikler GM, Gilbert JA, Pearson GR
Dept. Cell Biology, Section Biochemistry, Mayo Clinic, Rochester, MN, 55901
J Virol; 43(2):555-565 1982

A new class of Epstein-Barr virus nuclear antigen (EBNA) was identified by the complement fixation assay. This new species of EBNA is more tightly bound to chromatin and was termed Class II EBNA, as opposed to the more weakly associated species, Class I EBNA. Preparations of this new antigen(s) specifically reduced absorption with the titer of anti-EBNA antibodies as determined by the anticomplement immunofluorescence assay. Therefore, the complement fixation antigens (Class II EBNA) appear to be related to the classical EBNA (Class I EBNA). The Class I EBNA was found to focus at the same pH (4.6) as the soluble antigen found in the cytosol. The Class II EBNA differed from the Class I EBNA with regard to its overall charge, molecular size, antigenicity, and affinity for chromatin. The Class II EBNA appeared to be a basic protein, based on its apparent pI of 9.2 and its binding to cation-exchange resins. It differed from histones with regard to its molecular size (mol wt between 60,000 and 70,000) and its elution from hydroxylapatite chromatography. Steps were taken to prevent proteolysis and artifacts in the immunological assays and in the overall charge estimation of the new antigen by nonspecific basic histone protein-acidic protein interactions. Both Class I and Class II EBNA were identified by radioimmunoelectrophoresis on two-dimensional polyacrylamide gels with pI values of 5.0 and 8.5, respectively, and a mol wt range of 60,000 to 70,000 for both. A lower-mol-wt antigen identified by molecular sieve chromatography appeared to be due to interference by histones in the immunoassays since it was not observed by the two-dimensional gel electrophoresis. Further characterization of this Class II EBNA is in progress. (Author abstract) (40 Refs)

3. NON-HISTONE CHROMATIN PROTEIN METHYLATION IN NORMAL AND PROLIFERATING RAT LIVER AND IN HEPATOMA CELLS.

Kibert LG
University of Pittsburgh, Pittsburgh, PA, 15260
Diss Abstr Int [B]; 42(11):4271-B 1982

Non-histone chromatin protein methylation was examined in a number of proliferative model systems based on rat liver, as well as two transplantable Morris hepatomas. The study was aimed at determining whether alterations in methylation of these proteins might play a role in controlling liver and/or tumor cell proliferation. Methylation was estimated in vivo by injecting a double-labelled mixture of methionine (methyl-labelled 3H and internally labelled 14C) into the intact animal and calculating the ratios of incorporated radioactivity. Similar estimations were made in vitro using 3H methyl-labelled S-adenosyl methionine. Methylation of hepatic non-histone chromatin protein was found to be responsive to proliferative stimuli, insofar as these proteins acquired more radioactively labelled methyl groups in both in vivo and in vitro experiments. These observed increases occurred at specific times after the proliferative stimulus was administered; the vast majority of experiments employed either 70% hepatectomized or triiodothyronine injected animals. Higher-than-control-levels of nonhistone chromatin protein methylation appears also as a persistent characteristic in hepatoma tissues. The enhanced methylation appears, however, to be quantitative rather than qualitative in stimulated hepatic tissues: no causal link was established between this increased protein modification and hepatic cell proliferation per se. (no Refs)

4. DEFECTIVE DNA ENDONUCLEASE ACTIVITY ON ANTHRAMYCIN TREATED DNA IN XERODERMA PIGMENTOSUM AND MOUSE MELANOMA CELLS.

Lee DE, Okorodudu AO, Lambert WC, Lambert MW
Dept. Pathology, New Jersey Medical Sch., Newark, NJ, 07103
Biochem Biophys Res Commun; 107(1):395-402 1982

The endonucleolytic activities of nine nonhistone chromosomal proteins isolated from xeroderma pigmentosum (complementation group A) cells (XPA) and their normal counterparts, and from lymphoblastoid and Cloudman mouse melanoma cells, were assayed using anthramycin (AM)-treated circular duplex PM2 DNA. The binding of AM to DNA was determined spectrophotometrically. A DNA enodnuclease activity which was markedly increased against AM-treated DNA in the nonhistone chromatin protein of normal cells was absent from the nuclei of both XPA lymphoblastoid and Cloudman mouse melanoma cells. These results indicated the presence of an endonuclease with marked specificity for AM-DNA in normal human chromatin that is inactive in these other cell types. Other recent results have indicated that in XPA lymphoblastoid cells and mouse melanoma cells, this same chromatin-associated enzyme fraction has markedly increased activity against untreated, linear, duplex DNA. Thus, there may be more than one abnormality in this enzyme activity. (38 Refs)

CYTOPLASMIC AND CONTRACTILE PROTEINS

5. PHOSPHORYLATIONS OF THE SUBCELLULAR MATRIX IN CELLS TRANSFORMED BY ROUS' SARCOMA VIRUS.

Gacon G, Gisselbrecht S, Piau JP, Fiszman MY, Fischer S
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Eur J Biochem; 125(2):453-456 1982

The transforming protein of Rous sarcoma virus (RSV) is a phosphoprotein of Mr 60,000 (pp60src) which displays protein kinase activity specific for tyrosine residues; pp60src is associated with the plasma membrane and is recovered in the detergent-insoluble material which represents the subcellular matrix of the cell. After phosphorylation of this material of RSV-transformed cells with [gamma-32P]ATP, five phosphoproteins have been detected which are not seen in normal cells. These proteins (Mr = 135,000, 125,000, 75,000, 70,000, 60,000) contain phosphotyrosine. Their phosphorylation is strongly inhibited by anti-pp60src antibodies. In cells transformed by a temperature-sensitive mutant of RSV, these phosphoproteins, present at the permissive temperature, are no longer detected at the non-permissive temperature. It is concluded that these phosphorylations are mediated by pp60src protein kinase activity. This supports a possible role of the phosphorylation of cytoskeletal proteins in the transformation process. (Author abstract) (19 Refs)

6. PROTEIN CARBOXYMETHYLTRANSFERASE (PCM) IN C-1300 MURINE NEUROBLASTOMA (MNB) CELLS (MEETING ABSTRACT).

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Pediatr Res; 16(4,part2):128A 1982

PCM has been identified in a variety of tissues derived from neural crest anlage including in vivo MNB. These observations have stimulated inquiries regarding the role of PCM in tumor growth and differentiation. A tissue culture line derived from MNB has been used to characterize the subcellular distribution and kinetic behavior of PCM. The activity of PCM was determined in subcellular fractions of MNB cells after differential centrifugation. In the presence of exogenous substrate (+ gelatin), 51% of the PCM activity was present in the 100,000 x g supernatant and 28.8% in the 800 x g particulate fraction. Endogenous (- gelatin) activity, which reflects the relative amount of PCM and its methyl acceptor proteins, was low amounting to less than 2.6% and 0.5% of the total PCM activity present in the 800 x g particulate and 100,000 x g soluble fractions, respectively. Cytoplasmic PCM was competitively inhibited by adenosyl homocysteine, sinefungin and A9145C. These data demonstrate that the PCM activity of cultured MNB cells is located primarily in the soluble fraction similar to in vivo MNB and non-malignant neural tissues. Cultured MNB, however, exhibits lower endogeneous PCM activity and a higher Km and Vmax than PCM derived from in vivo MNB. (no Refs)

7. CUTANEOUS TUMOUR INVASION: CHANGES IN ACTIN EXPRESSION AND EPIDERMAL ANTIGENS (MEETING ABSTRACT).

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Pathology; 14(1):101 1982

To analyze the mechanism(s) of cutaneous tumor invasion, malignant and benign lesions were examined with pemphigus, bullous pemphigoid, and anti-actin antibodies using indirect immunofluorescence. In normal epidermis the presence of pemphigus antigen parallels squamous cell differentiation and the basement membrane shows continuous uniform staining with bullous pemphigoid antibody. In contrast to resting cells actively migrating cells have a prominent network of actin-containing microfilaments which is demonstrated by anti-actin antibody. Squamous and basal cell carcinomas examined with these antibodies show loss of pemphigus antigen in invading cells and of bullous pemphigoid antigen at the tumor-stromal interface. However, both tumors demonstrate an increased expression of actin (squamous greater than basal cells). The anti-actin antibody shows strong cell outline staining of the invading tumor cells. In contrast the neoplastic intraepidermal squamous cells of Bowen's disease still retain pemphigus antigen, a third of lesions show a small increase in actin expression and the integrity of the basement membrane remains intact when stained with bullous pemphigoid antibody. Of the benign lesions, solar keratoses only show a focal loss of pemphigus antigen in areas of dysplastic cells. The keratoacanthoma demonstrates no major change in tissue antigens, except that the basement membrane is thicker than normal when stained with bullous pemphigoid antibody. Thus cutaneous malignant tumor invasion is characterized antigenically by increased expression of actin-containing microfilaments in poorly differentiated cells and loss of basement membrane antigen allowing migration of tumor cells into the dermal stroma. The molecular events underlying these changes from intraepidermal lesions to local tumor invasion are at present poorly understood. (no Refs)

8. PURIFICATION AND PROPERTIES OF INORGANIC PYROPHOSPHATASE OF RAT LIVER AND HEPATOMA 3924A.

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Cancer Res; 42(9):3526-3531 1982

Inorganic pyrophosphatase (EC 3.6.1.1) has been purified to electrophoretic homogeneity from the soluble portion of the cytoplasm of rat Hepatoma 3924A and rat liver. It has a specific activity of 600 to 700 umol inorganic orthophosphate liberated per min per mg protein at 25 C, a value in the same range as the highly purified enzymes from yeast and Escherichia coli. By all criteria applied, the hepatoma inorganic pyrophosphatase is identical with the liver enzyme. It is a dimer with subunits with molecular weights of approximately 30,000 to 33,000 and has a pH optimum of 7.4, a Km for pyrophosphate of 5 uM, and a Ka for Mg2+ of 0.03 with a pyrophosphate concentration of 0.2 mM. It is not inhibited by high Mg2+ concentrations up to 20 mM. Other metal ions such as Zn2+ and Ca2+ do not activate. Mn2+ activates to less than 10% that of Mg2+ at 0.6 mM and has no effect at 1 mM or higher. In the presence of optimal (4 mM) Mg2+ concentration, Ca2+, Mn2+, Hg2+, and F- at 0.2 mM inhibited strongly, but Zn2+ at 1 mM was not inhibitory. The enzyme had no phosphatase activity toward any of the purine or pyrimidine nucleoside mono-, di-, and triphosphates or toward p-nitrophenyl phosphate, beta-glycerophosphate, glucose 6-phosphate, or glucose 1-phosphate. Bromo- or iodoacetate at high concentration had no inhibitory effect, but p-chloromercuribenzoate and p-chloromercuriphenylsulfonate inhibited strongly at low concentration. The purified enzyme was very unstable but was protected markedly at or above the pH optimum of 7.4 by cysteine, dithiothreitol, and glutathione. (Author abstract) (38 Refs)

9. AN UNUSUAL TYPE OF CYTOKERATIN FILAMENT IN CELLS OF A HUMAN CLOACOGENIC CARCINOMA DERIVED FROM THE ANORECTAL TRANSITION ZONE.

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Differentiation; 22(1):25-40 1982

Epithelia-derived tumors (carcinomas) can be distinguished from mesenchymally derived tumors by the presence of intermediate-size filaments of the cytokeratin type, which usually coincides with the absence of other types of intermediate-sized filaments such as vimentin filaments. In the course of diagnostic examinations of human tumors, using immunofluorescence microscopy, we have come across a case of an unusual carcinoma (primary tumor and lymph node metastasis) positively stained not only with cytokeratin antibodies but also with immunoglobulins present in vimentin antisera. Therefore, this tumor, a cloacogenic carcinoma apparently derived from the rectal-anal transitional region, has been examined in greater detail using both immunofluorescence microscopy and immuno-electron microscopy as well as gel electrophoretic analysis of cytoskeletal polypeptides from total tissue and from microdissected nodules enriched in carcinoma cells. We have found that antisera raised against vimentin contained not only vimentin antibodies but also immunoglobulins which specifically bound to the largest cytokeratin component. We conclude that the unusual reaction of immunoglobulins present in vimentin antisera with cytokeratin filament bundles does not represent specific binding to vimentin in these carcinoma cells, but is due to a component obviously widespread in vimentin antisera which binds specifically to a cytokeratin present in this type of tumor but not in most other carcinomas. It is proposed that use is made in diagnostic examinations of vimentin antisera or affinity-purified vimentin antibodies that have been pre-absorbed on cytokeratin protein, in order to eliminate such disturbing reactions. (Author abstract) (84 Refs)

10. CORRELATION OF PREKERATIN PEPTIDES AND ULTRASTRUCTURE IN EPITHELIAL CELLS OF HUMAN SKIN TUMORS IN VIVO AND IN VITRO.

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Anticancer Res; 1(6):323-328 1981

Prekeratin peptides and ultrastructure in the human skin tumors Bowen's carcinoma (BC), basal cell carcinoma (BCC), and squamous cell carcinoma (SCC), and in normal epidermis (NE) were compared. Although BC was highly pleomorphic with abundant giant cells, desmosomes and massive bundles of tonofilaments were prominent throughout with heavily keratinized clumps in dyskeratotic areas. The poorly differentiated SCC exhibited mostly sparse fibrillar aggregates. BCC cultures exhibited cobblestone-like patterns of polygonal cells stratifying extensively at higher densities with large keratinized squames. SCC explants formed mainly monolayers, hardly and horny scales, and only faint filamentous structures. Besides the quantitative differences in prekeratin level, the reduction in av prekeratin size was most obvious when comparing cytoskeletal preparations of tumors and NE. The larger peptides prominent in NE (65,000-68,000 daltons) were virtually absent except in some samples of BC. In BCC explants, prekeratin profiles were almost indistinguishable from those in epidermal explants and the comparably high levels evidently exceeded those in tumors. In contrast, SCC explants maintained largely the expression of 'SCC-type' prekeratins at a similarly low level. Results thus demonstrated a significant reduction in prekeratin levels corresponding to the ultrastructurally observed degree of differentiation, and a marked decrease in av subunit size rendering prekeratin patterns similar to those of normal keratinocytes in culture. (22 Refs)

11. HUMAN CHORIONIC GONADOTROPIN: STRUCTURAL, BIOLOGIC, AND IMMUNOLOGIC ASPECTS.

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Semin Oncol; 9(2):163-173 1982

The chemical, biological, and immunological properties of human chorionic gonadotropin (HCG) are described. The glycoprotein nature of HCG, its primary structure, associated carbohydrate moieties, hormonal activity, its antigenic nature and the subsequent development of sensitive radioimmunoassays are among topics discussed. The development of such assay techniques has particular relevance in the clinical management of trophoblastic disease. (78 Refs)

12. MONOCLONAL ANTIBODY TO SPLEEN FOCUS-FORMING VIRUS-ENCODED GP52 PROVIDES A PROBE FOR THE AMINO-TERMINAL REGION OF RETROVIRAL ENVELOPE PROTEINS THAT CONFERS DUAL TROPISM AND XENOTROPISM.

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J Virol; 43(2):472-481 1982

Monoclonal antibodies which recognize a region common to Friend spleen focus-forming virus encoded gp52 and Friend mink cell focus-inducing viral gp70 were isolated. One such antibody from hybridoma 7C10 was tested extensively in immune precipitation and was found to react with a determinant on envelope gp70s of all mink cell focus-inducing, xenotropic, and amphotropic mouse retroviruses tested, but not with envelope gp70s of ecotropic viruses, including Friend, Moloney, and AKR murine leukemia viruses. Monoclonal antibody from hybridoma 7C10 precipitated a 23,000-mol-wt fragment, derived by V8 protease digestion of Friend mink cell focus-inducing gp70. This 23,000-mol-wt peptide was determined to derive from the amino terminus of the molecule. These results correlate well with other genetic data which indicate that endogenously acquired sequences of mink cell focus-inducing viruses are found at the 5' end of the envelope gene. (Author abstract) (49 Refs)

13. CHARGE MICROHETEROGENEITY OF THE MAJOR CAPSID PROTEIN OF POLYOMA VIRUS.

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J Virol; 43(2):456-464 1982

The behavior in isoelectric focusing of the major capsid polypeptide VP1 of several strains of polyoma virus was studied. Two previously recognized phenomena were reexamined, namely, (i) the separation of the VP1 polypeptide into multiple subspecies differing only slightly from each other in apparent isoelectric point and (ii) strain differences in the overall apparent net charge of the family of VP1 subspecies. It was found that the pattern of subspecies was reproducible when focusing was initiated from either the basic or acidic region of the gel, keeping the ampholyte mixture constant. However, individual subspecies were unstable, and labeled polypeptide could be shifted dramatically by either refocusing of separated subspecies or by altering the concentration of ampholytes. These findings suggest that protein-protein and protein-ampholyte interactions play an important role in the generation of this charge heterogeneity. The basis for the overall charge difference between the VP1 of 3049 virus and several other strains (lpD, lpS, ts59, and A2) was studied, using recombinant viruses constructed of specific sequences derived from 3049 and lpD genomes. The portion of the VP1 polypeptide carrying the altered charge could be mapped to the body of the molecule 3' to the HindIII site at 45.0 map units (3,918 base pairs). This clearly segregates the VP1 charge phenotype from the cyc phenotype of 3049 in which capsid proteins are overproduced and accumulate in the cytoplasm of infected cells. (Author abstract) (32 Refs)

14. FUSION INJECTION OF ROUS SARCOMA VIRUS PROTEINS INTO ROUS SARCOMA VIRUS-TRANSFORMED, NON-PRODUCING HAMSTER CELLS CAUSES RELEASE OF INFECTIOUS VIRUS.

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J Gen Virol; 61(part1):83-86 1982

Purified virus proteins from transformation-defective (td) mutants of Rous sarcoma virus PrA or PrB were trapped in human erythrocyte ghosts which, after resealing, were fusion-injected into hamster RBH cells or rat TWERC cells. These cell lines are non-productively transformed by subgroup C Rous sarcoma virus. After fusion injection the hamster RBH cells released transforming subgroup C Rous sarcoma virus. No infectious virus could be rescued from rat TWERC cells. Since previous experiments have shown that fusion injection of the purified Rous sarcoma virus protein p15 into hamster RBH cells caused cleavage of the precursor protein pr76 to form the virus group-specific antigen (gag) but did not induce infectious virus, we conclude that in addition to p15 other virus proteins are required to induce virus rescue in hamster RBH cells. (Author abstract) (10 Refs)

PLASMA AND EXTRACELLULAR PROTEINS AND TUMOR PRODUCTS

15. PROTEIN SECRETIONS IN HAMSTERS WITH PANCREATIC CARCINOMA.

Allan BJ, White TT, Reber HA, Roberts C, Schilling JJ
Dept. Surgery RF 25, Univ. Washington, Seattle, WA, 98195
Dig Dis Sci; 27(5):437-443 1982

Pancreatic secretions from the hamster model for pancreatic ductal adenocarcinoma were analyzed to determine whether alterations had occurred in the protein composition. CCK- and secretin-stimulated secretions were collected from 13 animals with cancer and 16 normal controls. Subsequent separation of the proteins by isoelectric focusing showed the following: (1) Significant changes occurred in the protein composition from animals with carcinoma. An unusually dark band was present at pH 7.5 just below amylase (pH 7.65); two unidentified bands, present in the normals at pH 6.9 and 7.0, were missing; and marked decreases occurred in the cathodic proteins with isoelectric points above pH 9. (2) CCK provided the optimal stimulus for differentiating specimens from animals with carcinoma from the normal controls. (3) The protein concentrations of CCK-stimulated secretions of animals with carcinoma were significantly lower than the controls. We have concluded that the protein alterations which have occurred in the hamster model for pancreatic ductal adenocarcinoma warrant further investigation. (Author abstract) (13 Refs)

16. CHARACTERIZATION OF HIGH MOLECULAR WEIGHT CALCITONIN- AND ACTH-IMMUNOREACTIVE PROTEINS PRODUCED BY SMALL CELL LUNG CANCER TUMOR CELLS (MEETING ABSTRACT).

Gropp C, Luster W, Havemann K
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The Endocrine Lung in Health and Disease, June 2-4, 1982, Washington, D.C. The George Washington University Medical Center 31 pp., 1982.

In sera of about 200 patients with lung cancer, the peptide hormones calcitonin, ACTH, beta-lipotropin, and beta-endorphin were determined by radioimmunoassay: 57% of the patients with small cell carcinoma and 10% of the patients with squamous cell carcinoma exhibited elevated calcitonin levels. ACTH and beta-endorphin were increased in 33% and 45%, respectively, of patients with small cell carcinoma. In order to investigate the biochemical nature of these peptides, high molecular weight polypeptide fractions obtained from sera, tumor tissues and media of long term cell cultures, were separated by gel chromatography. Three immunoreactive calcitonin proteins with molecular weights of 100,000, 50,000 and 20,000 were isolated from all three sources by gel filtration and polyacrylamide gel electrophoresis under non-denaturing conditions. Two of the high molecular weight calcitonin fractions were degraded to molecular weights of about 17,000 and 3,400 (molecular weight of monomeric calcitonin) by incubation with sodium dodecyl sulphate. Moreover, the calcitonin-immunoreactive proteins were characterized by their behavior in ion exchange chromatography. Two fractions, with the molecular weights of 50,000 and 20,000, bound to DEAE (diethylaminoethyl)-sephacel at neutral pH and low conductivity and eluted at slightly acid pH or in the presence of 55 nM NaCl. The fraction having a molecular weight of 100,000 did not bind to DEAE-sephacel. None of the three protein fractions exhibited any interaction with a cation exchange gel. Additionally, the fraction having a molecular weight of 100,000, in contrast to the other two polypeptides, is characterized by a high sensitivity to proteolytic enzymes. Gel filtrations of cell culture media resulted in big-ACTH-beta-lipotropin fractions with molecular weights of about 150,000, 30,000 and 20,000 and only a small amount of monomeric ACTH. In summary, the different lung tumors seem to synthesize a corticotropin-beta-lipotropin precursor molecule and/or a calcitonin prohormone. (no Refs)

17. HUMAN LUNG SMALL-CELL CARCINOMA: THE AMPHIBIAN PEPTIDE CONNECTION (MEETING ABSTRACT).

Lazarus LH, DiAugustine RP, Jahnke GD
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The Endocrine Lung in Health and Disease, June 2-4, 1982, Washington, D.C. The George Washington University Medical Center 31 pp., 1982.

Among the different histopathologic classes of human lung tumors, small cell carcinomas (SCC) are considered to produce the highest incidence of clinical ectopic endocrine disorders. Many of these endocrinopathies are caused by the release of peptide hormones from constituent cells of the tumor. The presence of cytoplasmic neurosecretory-type granules in some SCC cells indicates a morphological correlate with endocrine cells. Recently, immunoreactivity (ir) to the amphibian peptides bombesin and physalaemin was found in extracts of a human SCC passaged in athymic (nude) mice. In the present study, the physicochemical properties of SCC bombesin-ir and physalaemin-ir were compared with those of the corresponding known amphibian peptides by reverse-phase HPLC (high performance liquid chromatography) following selected chemical and enzymic modifications. In each case, the retention times of tumor-ir were identical to that of the synthetic peptide: (1) increased retention of bombesin-ir by a change in pH of the mobile phase from 2.3 to 7.0 was attributed to the loss of charge on His 12, thereby increasing hydrophobicity; (2) oxidation of a thiol-containing amino acid in both bombesin-ir and physalaemin-ir produced elution patterns which suggested the presence of Met; (3) removal of the NH2-terminal less than Glu of bombesin-ir by pyroglutamate aminopeptidase produce a more hydrophobic molecule; and (4) digestion of bombesin-ir by trypsin yielded a peptide fragment that eluted earlier than the standard peptide. The increased retention of this fragment at pH 7.0 suggests the presence of a His residue. In addition, the threshold dose of tumor physalaemin-ir (HPLC-purified) required to produce a contractile response in the isolated guinea-pig ileum was similar to that of the amphibian peptide. (no Refs)

18. INHIBITION OF CELL-MEDIATED IMMUNITY IN SMALL-CELL LUNG CANCER (SMLC): CHARACTERIZATION OF MIDDLE-MOLECULAR-WEIGHT SERUM FACTORS TRANSFERRING THE DEFECT ONTO NORMAL HUMAN PERIPHERAL T-LYMPHOCYTES (MEETING ABSTRACT).

Manke HG, Burrow-Meckbach P, Bunzli I, Drings P, Toomes H
Klinik fuer Thoraxerkrankungen der LVA Baden, Tumorzentrum Heidelberg-Mannheim, Heidelberg, W. Germany

The Endocrine Lung in Health and Disease, June 2-4, 1982, Washington, D.C. The George Washington University Medical Center 31 pp., 1982.

Low T-cell counts (E-rosette forming cells) are a prominent feature in patients with SMLC, and there is a parallel inhibition in vivo as well as in vitro of cell-mediated immunity in these patients. More than 50 per cent of 57 patients with SMLC showed low E-rosette forming capacity. Sera of patients with low E-rosette counts as well as serum factors prepared by medium-molecular-weight HPLC (high performance liquid chromatography) transferred the inability to form E-rosettes onto normal peripheral human lymphocytes, especially those T-lymphocytes with a high turnover of the 50,000 dalton membrane glycoprotein, which is mediating the binding between erythrocyte and T-lymphocyte. The molecular weight range of the factors is between 15,000 and 45,000 daltons. They lower or inhibit protein synthesis. The origin of these factors is discussed: it is possible that they might be products of the tumor, products of the lung environment reacting against the tumor, or products of the local defense of the immune system against the tumor. Serum of patients without low E-rosette counts do not contain these inhibiting substances. (no Refs)

19. PULMONARY CARCINOIDS: IMMUNOHISTOCHEMICAL DEMONSTRATION OF BRAIN-GUT PEPTIDES (MEETING ABSTRACT).

Yang K, Ulich T, Taylor I, Cheng L, Lewin K
Department of Surgical Pathology, University of California at Los Angeles Hospital, Los Angeles, CA

The Endocrine Lung in Health and Disease, June 2-4, 1982, Washington, D.C. The George Washington University Medical Center 31 pp., 1982.

The purpose of this study was to investigate the presence and distribution of neuroendocrine products within bronchial carcinoid tumors. Antisera raised against serotonin, gastrin, somatostastin, motilin, bombesin, calcitonin, secretin, glucagon, ACTH, neurotensin, and pancreatic polypeptide carboxyterminal hexapeptide were employed to immunohistochemically stain seven pulmonary carcinoids. Argentaffin and argyrophil stains were also performed on all cases. Serotonin-like immunoreactivity was present in four tumors, pancreatic polypeptide-like immunoreactivity in four tumors, bombesin-like immunoreactivity in two tumors, and ACTH-like immunoreactivity in one tumor. The cells shown to contain neuroendocrine products constituted a minority cell population in all tumors except the ACTH-immunoreactive tumor. Five tumors were argyrophilic, and one was argentaffinic. The non-neoplastic respiratory epithelium overlying one tumor contained bombesin-like immunoreactivity, but otherwise no immunoreactivity was noted in the respiratory mucosa. The patient whose tumor demonstrated ACTH-like immunoreactivity presented clinically with Cushing's syndrome, and his tumor contained a predominance of ACTH-secreting cells. This study suggests that pulmonary carcinoids, like their abdominal counterparts, contain a variety of neuroendocrine products and may produce more than one neuroendocrine product. Serotonin and pancreatic polypeptide-like immunoreactivity were the most prevalent neuroendocrine products demonstrable in the present study. (no Refs)

20. ACUTE PHASE REACTANT PROTEINS IN DIAGNOSIS OF PATIENTS WITH COLORECTAL CANCER (MEETING ABSTRACT).

Uehara Y, Kojima O, Kurimoto T, Yamane T, Kasuga M, Watanabe S, Nishioka B, Majima S

Prefectural University of Medicine, Kyoto, Japan

IXth Biennial Congress of the International Society of University Colon and Rectal Surgeons, June 6-10, 1982, Munich, W. Germany. Society of University Colon and Rectal Surgeons 175 pp., 1982.

Measurement of serum Carcinoembryonic antigen (CEA) level is useful in monitoring a recurrence of cancer, but may be of limited value in the diagnosis of new primary colorectal cancer. It has been shown that serum levels of acute phase reactant proteins (APRP) are elevated in patients with malignancies. In this study, two sugar moieties (Sialic acid and Hexose) of APRP and CEA were applied to the diagnosis of colorectal cancer. Serum CEA was determined using commercially available Roche kits. Serum sialic acid and hexose were determined using commerically available Roche kits. Serum sialic acid and hexose were determined by colorimetric methods. The values of serum sialic acid and hexose in colorectal cancer patients tested were 2.26 +/-0.12, umol/ml and 3.67 +/-0.19 mg/ml, respectively. Both values were significantly higher than in normal subjects (sialic acid: 1.70 +/-0.04 umol/ml, hexose: 2.45 +/-0.18 mg/ml) (P less than 0.001). The upper normal limit for CEA was determined to be 5.0 ng/ml. The upper normal limit for sialic acid and hexose were determined to be 1.96 umol/ml and 3.58 mg/ml respectively; according to mean of normal subjects. With these three variables, serum levels above the upper normal limits were defined as 'Positive'. Forty eight patients with colorectal cancer were classified into either 'Positive' or 'Negative' groups. In classifications using a single variable, the percentages of 'Positive' were as follows: sialic acid: 64% of patients, Hexose; 42%, CEA; 38%. If at least one of the three variables tested as positive, the patient was considered to be 'Positive' group. Our data suggests that the simultaneous measurement of serum sialic acid, hexose and CEA is useful for detection of colorectal cancer. (no Refs)

21. PURIFICATION OF CARCINOEMBRYONIC ANTIGEN BY AFFINITY CHROMATOGRAPHY WITH ANTI-ALPHA-ACID GLYCOPROTEIN.

Ochi Y, Fujiyama Y, Hosoda S, Hamazu M, Kajita Y, Myazaki T, Hachiya T, Ishida M
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J Immunol Methods; 52(2):213-221 1982

Purification of radiolabeled carcinoembryonic antigen (CEA) preparations by affinity chromatography with anti-AG bound to Sepharose wás attempted, since an immunological similarily between AG (alpha1-acid glycoprotein) and a portion of CEA had been noted. When 125I-CEA was purified in this manner, the fraction which did not bind to the column showed decreased reactivity with either anti-AG or anti-CEA. The retained fraction showed enhanced reactivity with both anti-AG and anti-CEA. The yield of purified CEA increases when the CEA preparation was allowed to react with the anti-AG column overnight. Purification of CEA from tumor tissue was performed by affinity chromatography. A perchloric acid (PCA) extract from cancer tissue was mixed with antiserum against CEA to give an immune complex, and a CEA-reactive fraction obtained by PCA extraction. The CEA-reactive fraction was eluted from a Sephadex G-200 column, and final purification was by anti-AG chromatography. When purified CEA was applied to a Sephadex G-200 column with carrier protein after labeling with 125I, the eluted radioactivity was found only in the 180,000 dalton fraction. Almost all the radioactivity was precipitated from the labeled protein by either anti-AG or anti-CEA. Purification of CEA is possible by affinity chromatography with anti-AG bound to Sepharose. (Author abstract) (6 Refs)

22. MICROHETEROGENEITY OF ALPHAFETOPROTEIN (AFP) IN DIAGNOSIS OF CANCER (MEETING ABSTRACT).

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Dept. of Pathological Anatomy, Academy of Medicine, Poznan, Poland
Fifth European Immunology Meeting, June 1-4, 1982, Istanbul, Turkey. Turkish Society of Immunology 354 pp., 1982.

By means of lectin-affino-electrophoresis microheterogeneity of AFP present in sera of 92 patients was studied. Thirty patients with yolk sac tumor (YST), 18 patients with hepatoma, 10 patients with liver metastases of cancer, 21 patients with liver tumors of unknown origin - preliminary clinical diagnosis: hepatoma and 17 patients with benign liver disorders. AFP was divided into two variants when Concanavalin A (Con A) was used and into three variants when Lentil agglutinin / LCA/ was used. Relative amounts of Con A and LCA, AFP variants varied and were the same in various groups of patients - listed above. Connected results obtained in Con A and LCA-affino-immunoelectrophoresis of a one sample were termed 'AFP profile'. In all 92 patients sera 9 main (coded: H1-3-hepatoma, Y1-4-YST, L - liver benign liver disorders, M - liver metastases of cancer) and 5 single (coded: A1-5-atypical) AFP profiles were demonstrated. Comparison of the obtained AFP profiles made possible differentiation of all patients with hepatoma, YST and patients with liver metastases of cancer. Moreover, 2/3 patients with hepatoma differed from patients with benign liver disorders. Six out of 21 patients with liver tumors of unknown origin had hepatoma and the remaining 15 patients had liver metastases of cancer. In conclusion: study of AFP variants is a new diagnostic tool for differential diagnosis of cancer in patients with elevated levels of serum AFP. (no Refs)

23. MACROPHAGE-AFFECTING SOLUBLE FACTORS FROM RAT TUMOR ASCITES (MEETING ABSTRACT).

Fahlbusch B, Zschiesche W, Schumann I
Zentralinstitut fur Mikrobiologie und Experimentelle Therapie, Jena, West Germany
Fifth European Immunology Meeting, June 1-4, 1982, Istanbul, Turkey. Turkish Society of Immunology 354 pp., 1982.

Wistar rats bearing Zajdela ascites hepatoma demonstrated the appearance of soluble factors with the following properties; (1) Inhibition of macrophage migration (MIF); (2) stimulation of macrophage migration; (3) activation of macrophages for reduction of the multiplication of intracellularly growing becteria (MGIF). Kinetic studies showed that the factors appear in the order referred to above. MIF and MGIF present as relatively heat stable (glyco)proteins. They demonstrate striking microheterogeneity with respect to (1) molecular weight as evidenced by gel filtration, (2) net charge as shown by means of ion exchange chromatography, and (3) isoelactric point as shown by isoelectric focussing. There is evidence that the factors are, at least in part, produced by the tumor cells themselves. It may be suggested that factors of the kind referred to above interfere with host defense mechanisms against tumors. (no Refs)

24. ELASTIN DEGRADATION BY PROTEASES ELABORATED BY CULTURED HUMAN BREAST CANCER CELLS (MEETING ABSTRACT).

Kao R, Wong M, Stern R
University of California, San Francisco, CA, 94143
Fed Proc; 41(3):637 1982

Potent elastinolytic activity was elaborated into the media of the cultured human breast cancer cell line ZR 75-31 A. This activity was resolved into three major peaks of activity by DEAE (diethylaminoethyl)-cellulose column chromatography. The three elastinolytic activities were clearly separated from the collagenolytic activity also produced by these cells. Inhibition profiles of the elastinolytic activities indicated that two were serine proteases and one was a metalloprotease. Each of these three proteases exist in a zymogen form and require trypsin pretreatment for activity. The scirrhous reaction to human breast cancer contains both collagen and elastin. The present findings indicate that the breast tumor cells may themselves be able to modulate the turnover of these stromal proteins. (no Refs)

25. PURIFICATION AND CHARACTERIZATION OF A PLASMINOGEN ACTIVATOR FROM RAT PROSTATE ADENOCARCINOMA CELL LINE PA-IIIF (MEETING ABSTRACT).

Strickland DK, Chan SY, Pollard M, Castellino FJ
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Fed Proc; 41(3):444 1982

A plasminogen activator secreted by a rat prostate tumor line, PA-IIIf, grown in a chemically defined medium (MB-DF) has been purified and partially characterized. Purification was accomplished by gel filtration on a Sephacryl S-200 column, followed by affinity chromatography on a Sepharose-benzamidine column. The activator was eluted from the Sepharose-benzamidine column with a buffer containing 2 M guanidine hydrochloride. These procedures resulted in a 360-fold purification and a total yield of 66%. The specific activity of the purified activator was 192,000 U/mg. The apparent molecular weight of the activator, determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, was approximately 52,000.

The purified activator was found to possess amidolytic activity toward the tripeptide substrate, D-Ile-Pro-Arg-p-nitroanilide (S-2288). Determination of the kinetic parameters indicated a Km of 0.33 mM and a Vmax of 0.327 nmoles/min-U. The kinetics of plasminogen activation were examined, using both human glu1-plasminogen and lys77-plasminogen. The Km(app) for glu1-plasminogen was 1.7 uM, with a Vmax of 0.555 pmoles plasmin/min-U, while the Km(app) for lys77-plasminogen was 0.47 uM, with a Vmax of 5.34 pmoles plasmin/min-U. The activator was demonstrated to be sensitive to DFP. At a concentration of 1 mM DFP, and 152 U/ml activator, the half-life was 3.8 minutes. (no Refs)

26. MURINE TUMOR NECROSIS-INDUCING FACTOR: PURIFICATION AND EFFECTS ON MYELOMONOCYTIC LEUKEMIA CELLS.

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JNCI; 68(6):997-1003 1982

The tumor necrosis-inducing factor (TNF) found in sera of Corynebacterium parvum-treated, endotoxin-stressed BALB/C and outbred albino CD-1 mice has been purified to a single band of protein by polyacrylamide gel electrophoresis after identification and removal of contaminating albumin and transferrin. This purified TNF has a molecular weight of 140,000, is glycoprotein in nature, and migrates of free electrophoresis as an alpha 2-globulin. TNF activity was continuously monitored during purification by bioassay in vitro (tumor cell types) and was confirmed by demonstration of induction of tumor necrosis in vivo. A single target tumor cell line, murine myelomonocytic leukemia (WEHI/3) was used in both assays. In the in vivo assay, controls were heat-inactivated samples of TNF. As additional controls, a line of TNF-resistant WEHI/3 cells was used in the in vitro assay. Results from in vivo radiolabeling of TNF-sensitive and TNF-resistant cells indicated a difference between their cytoplasmic peptide profiles. Optimal TNF production was not altered in C parvum-endotoxin-treated mice by treatment with silica, a substance that is specifically toxic for macrophages. Exposure of mice to 650 rad whole-body radiation, which is not markedly damaging to macrophage elements in the reticuloendothelial system, completely abrogated the ability of the mice to produce TNF after C parvum-endotoxic treatment. These findings suggest that in the sera of C parvum-endotoxin-treated mice the protein that induced necrosis in tumors may not be of macrophage origin. (Author abstract) (34 Refs)

27. A DISTURBANCE IN THE SERUM PROTEINASE INHIBITORS IN LEUKEMIC PATIENTS (MEETING ABSTRACT).

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Fed Proc; 41(3):1122 1982

Malignant cells secrete various proteinases which are involved in the metastasis of the tumor. We previously showed that a trypsin inhibitor EDC1 (Mr 27.5 K and immunologically related to a normal serum protein, inter-alpha-trypsin inhibitor, IATI) was excreted in large quantities in urine of metastatic cancer patients. We have now measured immunoreactive (IR) titers of urinary EDC1 and four major serum antiproteinases in 16 patients with hematologic tumors and 24 healthy normal subjects. The urinary EDC1 levels were significantly elevated in the cancer group as compared to the normals (273.4 vs 8.0 mg/g creatinine). Serum levels of IR-alpha 1 antitrypsin (AT) and alpha 1-antichymotrypsin (AC) were higher in the leukemic group (100 to 150% increase), IR alpha 2-macroglobulin levels were unchanged while IR-IATI levels were depressed (63% of the normal). There was a negative correlation between urinary EDCl and serum IATI levels. The increased levels of IR-AT were not reflected in the total trypsin inhibitory capacity of cancer sera which was significantly lower (p less than .05) than that for the normal group. It suggests that cancer may accelerate synthesis of AT and AC, which may exist either as complexes with enzyme(s) or as degradation products which retain their immunoreactivity but are no longer inhibitory. (no Refs)

28. BEHAVIOR OF ANTITHROMBIN III, DETERMINED AS PROTEIN CONCENTRATION AND BIOLOGIC ACTIVITY, IN SUBJECTS WITH NEOPLASTIC DISEASE.

Pengo V, Guerra C, Cartei G, Fiorentino M
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Tumori; 68(3):205-209 1982

Antithrombin III (AT III), the primary inhibitor of plasma protease coagulation proteins, was evaluated in groups of patients with different neoplastic disease. When compared with that of 25 healthy subjects, the mean value of AT III biologic activity was elevated in all groups, significantly in gastrointestinal (p less than 0.02) and lung (p less than 0.001) tumors, lymphoma (p less than 0.02) and in the group with various primary cancers (p less than 0.05). No difference was found between 46 patients tested under chemoradiotherapy and 216 patients who had not undergone therapy for at least 1 mo. Four patients with clinical thrombosis had normal AT III biologic activity. Of the 262 patients studied, 14 had low AT III biologic activity without clinical thrombosis and normal protein concentration. In 71 patients (27%) an excess of immunoassayable protein of 20% or more over biologic activity was found. Double immunoelectrophoresis performed in five of these showed for three patients an enlarged second arc of precipitation. Moreover, no correlation was observed between the activity and protein AT III concentration ($r = 0.05$). The likelihood of the presence of circulating AT III-protease complexes is discussed. (Author abstract) (17 Refs)

29. COMPARISON OF 125I-FIBRINOGEN KINETICS AND FIBRINOPEPTIDE A IN PATIENTS WITH DISSEMINATED NEOPLASIAS.

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Blood; 60(2):381-388 1982

To provide more information on the pathways of fibrinogen catabolism in generalized cancer, the effect of heparin on fibrinopeptide A (fpA) and on 125I-fibrinogen kinetics was studied in 15 patients with disseminated neoplasia. Three patients had evidence of venous thrombosis and in two additional patients a low fibrinogen level together with increased amounts of FDP/fdp and a positive ethanol test indicted disseminated iv coagulation (DIC). The plasma levels of fpA were grossly elevated (4.6-20, mean 11.4 ng/ml, normal values 1.01 +-0.45 ng/ml) in patients with thrombosis or DIC, and normal to grossly elevated (0.4-10.4, mean 6.1 ng/ml) in the other patients. Iv heparin bolus lowered the fpA level in 11/11 patients, and continuous heparin treatment led to an impressive suppression or complete normalization of the plasma fpA in 5/6 patients. This finding is thought to reflect heparin suppression of thrombin activity on fibrinogen. In some cases, the fpA fall after heparin bolus was slow and/or incomplete, suggesting fpA generation at sites not easily accessible to heparin or insufficient heparin dosage. The 125I-fibrinogen kinetics were characterized by a significantly shorter half-life (t1/2: 2.5 days), increased catabolic rate constant (j: 0.44 days-1), and increased absolute turnover (68.9 mg fibrinogen/kg/day) as compared to four normal subjects (t1/2: 4.2 days; j: 0.26 days-1; turnover 21.7 mg fibrinogen/kg/day). As estimated from the fpA generation rates, intravascular thrombin action on fibrinogen contributed only in minor part to increase the turnover of 125I-fibrinogen. In particular, the turnover was greatly accelerated in heparin-treated patients despite impressive suppression or normalization of the fpA levels in 5/6 cases. (Author abstract) (27 Refs)

30. LYSOSOMES IN INVASIVE LARYNGEAL CARCINOMA.

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Onkologie; 4(2):88-92 1981

The occurrence, distribution, morphology and enzymatic activity of lysosomes in malignant keratinocytes of laryngeal carcinoma was studied by electron microscopy and ultrastructural cytochemistry. Lysosomes can be observed in various forms: dense bodies, multivesicular bodies, myelin bodies and residual bodies. The acid phosphatase activity varied both with regard to intensity and staining pattern. Specific reaction product in lysosomes is present in the cell cortex and in cytoplasmic processes of basal carcinoma cells. Extracellular localization of acid phosphatase was also observed at the tumor-stroma junction. The enzyme cytochemical and morphological results are discussed in relations to current concepts of lysosomal enzymatic activity in tumor cells. The findings suggest, that extracellular secretion of lysosomal enzymes may play an important role in facilitating tumor invasion and metastasis. (Author abstract) (23 Refs) (In GER)

31. COLLAGENOUS ACTIVITY OF NEOPLASTIC AND RAPIDLY PROLIFERATING NORMAL TISSUES.

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Onkologie; 4(1):30-32 1981

By means of a biological collagenase assay the activity of this protease was examined in 12 invasive adenocarcinomas of the colon and compared to that in 10 normal thymus tissue biopsies. Collagenolytic activity at dimensions of 10(-3) units/ mm O tissue could be revealed in all carcinomas, which significantly (p less than 0.001) surpassed the also regularly detectable activity in thymi. While O-Phenantrolin and D-Penicillamin totally inhibited lysis of each sample, EDTA and normal human serum, remarkably inhibiting collagenase of normal thymus, only were able to reduce the lysis by cancer at 19 and 23% in the av leading to enzyme:inhibitor imbalance in malignancy. It can be suggested that collagenolysis displayed by adenocarcinomas is derived from the tumor itself, and is different from that of normal thymus - a rapidly proliferating organ - by means of enzymatic quantity and quality. (Author abstract) (18 Refs) (In GER)

32. CONTROL OF CELL GROWTH BY TISSUE AND SERUM FACTORS.

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Diss Abstr Int [B]; 42(11):4368-B 1982

KCl extracts from rat kidney, rat liver, and Morris hepatomas inhibited 3H-thymidine incorporation into cultured cells. The most pronounced inhibition was achieved with the kidney extract. A normal serum protein disappears from the serum of animals bearing Morris hepatomas 7777 and 5123 as the tumors increase in size. Serum factor levels are increased well over those found in normal rats 24 hours after partial hepatectomy. Increased levels persist for 12 days following partial hepatectomy. The serum protein is also increased following injections of T3 (triiodothyronine) and is decreased in thyroidectomized animals. Elevated levels of T3 are correlated with elevated levels of the serum protein and both are correlated with increased hepatic DNA synthesis. Low levels of T3 as a result of thyroidectomy are followed by low levels of serum protein resulting in a diminished capacity for hepatic DNA synthesis and decreased hepatoma growth. T3 injections restored the capacity for hepatic DNA synthesis and hepatoma growth. Injection of purified serum factor into normal rats resulted in increased 3H-thymidine incorporation in liver cells. Hepatoma growth was higher in rats that had elevated serum factor levels at the time they received a hepatoma transplant. It is suggested that the synthesis of this serum protein is stimulated by T3 and hepatocyte proliferation and hepatoma growth are influenced by the serum protein. (no Refs)

33. PURIFICATION AND PARTIAL CHARACTERIZATION OF A BONE-RESORPTIVE PROTEIN FROM CANCER ASCITES FLUID.

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Diss Abstr Int [B]; 42(6):2341-B 1982

A protein capable of stimulating bone-resorption in vitro has been purified approximately 1250-fold from cancer ascites fluid. An apparent molecular weight of 43,000 daltons was calculated. In terms of its amino acid composition, the bone-resorptive protein is distinguished by its relatively high content of glycine (17%) and proline (11%). Initial biological characterization of the purified ascites protein demonstrated that the protein is as potent as parathyroid hormone in its ability to stimulate bone-resorption in vitro. Both proteins stimulated the release of radioactivity into the medium from bones which were previously labeled with calcium-45 or tritiated proline indicating that both matrix and mineral were solubilized. However, the bone-resorptive protein present in cancer ascites fluid can be distinguished from parathyroid hormone. The molecular weight of the protein purified in this study is approximately four times that of parathyroid hormone and the ascites protein contains no methionine while this amino acid is present in parathyroid hormone. Also, in contrast to parathyroid hormone, stimulation of isolated bone cells with the ascites protein did not increase cAMP levels. Moreover, although parathyroid hormone and the ascites protein increased calcium uptake in isolated bone cells, only the ascites bone-resorptive protein stimulated increased calcium uptake in isolated skin cells. (no Refs)

34. ISOLATION OF A HYBRIDOMA CELL LINE WITH SPECIFICITY FOR HUMAN LP(A) (MEETING ABSTRACT).

Duvic CR, Smith GM, Sledge WE, Murray MD, Roheim PS, Gallaher WR, Thompson JJ

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Fed Proc; 41(3):1122 1982

A hybridoma cell line, IIIC9, was derived by fusion of mouse myeloma cells with spleen cells from BALB/c mice immunized with HDL (high density lipoprotein) (d equals 1.063-1.21 g/ml) from human serum. The antigen detected by IIIC9 was selectively enriched in the d equals 1.050-1.080 g/ml serum fraction and was essentially absent in fractions of d less than 1.050 and d greater than 1.21 g/ml. Antibody reactivity was confined to a lipoprotein distinct from LDL (low density lipoprotein), VLDL (very low density lipoprotein), and HDL and was localized to a pre-beta electrophoretic band in agarose. The particle containing the antigen was purified by gel filtration chromatography on agarose. This particle was found to contain apolipoprotein B (apo B) as well as the antigen detected by IIIC9. Since these properties were consistent with those described for Lp(a), comparisons were made with rabbit reference antiserum to Lp(a). The correlation coefficient between inhibition enzyme immunoassays, done with either the reference rabbit antiserum as detection reagent or the mouse IIIC9 antibody as detection reagent, exceeded 0.98. We conclude that the monoclonal antibodies produced by hybridoma IIIC9 detects an antigen localized on, or closely associated with, Lp(a). (no Refs)

35. IDENTIFICATION OF PANCREATIC-LIKE RIBONUCLEASES IN HUMAN BODY FLUIDS FROM PATIENTS WITH CANCEROUS AND NONCANCEROUS PANCREATIC DISORDERS (MEETING ABSTRACT).

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Fed Proc; 41(3):1205 1982

A protein-specific radioimmune assay (RIA) was developed using antibodies against purified human pancreatic ribonuclease. The RIA is sensitive in the range of 0.2-200 ng of RNase, roughly equivalent to our enzymic procedures and sufficient to quantitate RNase in 2 ul of serum or urine. Antisera completely inhibit purified RNase activity as well as activity in pancreatic extracts. Gel filtration of pancreatic extract showed a single activity peak and a nearly coincident peak in the RIA. To test the controversial proposal that circulating levels of pancreatic RNase are elevated in cases of pancreatic carcinoma, we have used immunological methods to identify and quantify human pancreatic-like RNases. Equivalent amounts of antisera inhibit RNase activity in serum and urine by 60-75%. Gel filtration of normal human serum showed two adjacent RNase activity peaks in which about 95% (major peak) and less than 40% of the activity cross-reacted in the RIA. These results suggest that most but not all of the RNase in these fluids is structurally related to the pancreatic protein. RNase activity measurements of serum from patients with histologically confirmed pancreatic carcinoma showed a 2-3 fold increase over serum from normal individuals. This result was confirmed exactly in the RIA and suggests that the additional serum RNase in patients with pancreatic cancer originates from the pancreas. (no Refs)

36. A NEW VASOACTIVE PEPTIDE RELEASED FROM RAT PLASMA KININOGEN BY A RODENT TUMOR ACID PROTEASE (MEETING ABSTRACT).

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Fed Proc; 41(3):1474 1982

This study reports on the purification and characterization of a new vasopeptide formed by the action of a Murphy-Sturm lymphosarcoma (MSLS) acid protease on rat plasma kininogen. Purified rat plasma kininogen (116 mg) was digested with 10 units of purified MSLS tumor acid protease in 0.05 M citrate-phosphate buffer, pH 3.0 for 16 hr. The released polypeptides were fractionated on Sephadex G-50 column. Smooth muscle stimulating activity was monitored on isolated rat uterus. The peptide purified further on a CM (carboxy methyl)-cellulose column showed a single ninhydrine positive spot (Mgly 2.1) on high voltage electrophoresis. Amino acid analysis revealed 2 Ser, 4 Pro, 1 Gly, 1 Ile, 1 Leu, 2 Phe and 2 Arg per M of peptide. The amino acid and carboxyl termini were identified as Ile and Leu respectively by dansyl chloride procedure and by treatment with carboxypeptidase Y. Tryptic and chymotryptic digestion products of polypeptide have been resolved by high voltage electrophoresis on thin-layer cellulose plates and complete sequence of these peptides is being elucidated. (no Refs)

37. MURINE B LYMPHOMAS: MODELS FOR IMMUNOGLOBIN EXPRESSION IN B CELL DEVELOPMENT.

Word CJ

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Diss Abstr Int [B]; 42(9):3582-B 1982

Individual clones of three B lymphoma cell lines (A20-1.11, X16C-4.3, K46-056) were found to synthesize two intracellular species of gamma 2a heavy chain which differ in apparent size by approx 6,000 daltons. The smaller gamma 2a heavy chain species is secreted by the cell and the larger gamma 2a heavy chain species is localized on the external cell membrane. Two species of mRNA, which apparently encode the two gamma 2a heavy chain species, were detected in the cytoplasm of the cells. It appears that the smaller (2.0 kb) mRNA species codes for the secreted gamma 2a heavy chain and the larger mRNA species (4.0 kb) codes for the membrane gamma 2a heavy chain. Fusion of the A20-1.11 or X16C-4.3 B lymphoma to myeloma cell lines results in hybrids expressing the more differentiated myeloma phenotype. That is, the expression of the secreted form of the lymphoma gamma 2a heavy chain is amplified as much as 150 fold while the expression of the membrane form of the lymphoma gamma 2a heavy chain polypeptide and corresponding mRNA is greatly diminished or abolished in the hybrid. Less than five percent of normal B lymphoid cells or plasma cell tumors express lambda light chains, and only rare tumors have been described that simultaneously express both lambda and kappa light chains in a single cell. However, three cloned B lymphoma cell lines (K46-056, B17-1.11, X16C-4.3) were found to express both lambda and kappa light chains. Different patterns of assembly of the light chains to the heavy chains were found. (no Refs)

38. IMMUNOGLOBULIN (IG) FREE LIGHT CHAIN SYNTHESIS IN NON HD LYMPHOMA: A MARKER OF B CELL DIFFERENTIATION (MEETING ABSTRACT).

Smith JL, Hannam-Harris AC, Gordon J, Wright DH

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Fifth European Immunology Meeting, June 1-4, 1982, Istanbul, Turkey. Turkish Society of Immunology 354 pp., 1982.

Studies investigating the biosynthesis of immunoglobulin (Ig) by cells from B cell chronic lymphocytic leukemia (CLL), non Hodgkin's lymphoma and leukemic reticuloendotheliosis have noted differences in the Ig light chain (LC) and heavy chain (HC) composition of secreted Ig with a strong consistent correlation between immaturity defined by the class and intensity of SIg expression and the synthesis and secretion of free LC by cells in culture. Free LC secretion has been noted in cultures of subpopulations of normal adult spleen cells and fetal liver lymphocytes and appears to be associated with early stages of B cell development. Furthermore, pokeweed mitogen stimulation induced balanced synthesis of HC and LC in CLL and normal spleen subpopulation after 6 days in culture. This study examines the link between free LC secretion and B cell immaturity in non HD lymphoma using the Kiel developmental scheme for lymphoma classification to define the relative maturity of the tumor cells. Twenty-four cases of non HD lymphoma have been studied. In the small lymphocytic lymphoma group secreted Ig consisted of LC exclusively or in excess over HC. Lymphomas of follicular center-cell origin considered to arise from cells further along the normal B cell differentiation pathway, can be divided into centroblasts or centrocytes according to their histological appearance in tissue sections. Centroblastic lymphomas exhibited strong SIg M or SIg G expression and the secretion of whole Ig was higher than cells from the small lymphocytic lymphomas. Synthesis of HC and LC was balanced in these cultures both intracellularly and in secreted material. The centrocytic lymphomas comprised a functionally more heterogeneous group, the SIg varied in intensity and was of SIg M, D or G class. Likewise Ig synthesis was variable in quantity and composition, some cases secreting LC exclusively while others including the cases expressing SIg G, secreted balanced HC and LC. In the Kiel classification centrocytes are considered to be more mature than centroblasts. Our data suggest that centrocytic lymphomas are heterogeneous in orgin some preceding and others following centroblasts in the B cell maturation sequence. These data are discussed in relation to current concepts of B cell 10/2 maturation and lymphoma histology. (no Refs)

39. SHED PROTEINS IN MALIGNANT MELANOMA. (REVIEW).

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Anticancer Res; 1(6):313-316 1981

Shed antigens, melanoma specific proteins, specificity, molecular nature, and interrelationship of melanoma antigens, and host immune response are reviewed. The synthesis of abnormal proteins has been shown to occur both in human and in murine melanoma cells in vivo and in vitro. The mechanism whereby the antigens are produced is not understood, although they differ from normal cellular products by having one or more deleted sequences of amino acids internally. The new sequences are probably responsible for the ultimate specificity of the novel proteins. (36 Refs)

40. THE CHARACTERIZATION OF ALPHA-SUBUNIT OF GLYCOPROTEIN HORMONE PRODUCED BY UNDIFFERENTIATED CARCINOMA.

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Endocrinol Jpn; 29(1):11-19 1982

The glycoprotein alpha-subunit (gp-a) of human chorion gonadotropin (HCG) was isolated from the urine of a 32-yr-old woman patient with an undifferentiated, metastatic carcinoma of the femur that produced large amounts of gp-a. The gp-a was compared with gp-a from normal pregnant women. The carcinoma did not produce complete HCG molecules or HCG-beta subunits (HCG-b). The specific immunoactivity of gp-a was 0.92 (mg alpha-subunit/mg protein) after extraction by the kaolin-acetone method. Gp-a showed identical immunoantigenicity to that of standard gp-a in the homologous HCG-alpha radioimmunoassay. Disc electrophoresis demonstrated a mol wt of 18,000 daltons for tumor gp-a, while standard HCG consisted of two components with mol wt of 19,000 and 28,000 daltons. Tumor gp-a showed close similarity to standard gp-a in amino acid composition. Tumor gp-a contained slightly more aspartic acid, serine, glutamic acid, and glycine and slightly less half-cystine and methionine than standard gp-a. In isoelectric focusing tests, gp-a produced by the tumor or by normal pregnant women was distributed over the range pH 3.5-6.0, while standard gp-a was distributed over the range pH 6.0-8.0. Tumor gp-a did not combine in vitro with HCG-b. Results suggest that tumor gp-a is identical with standard gp-a in the protein moiety but has undergone intracellular modification of the carbohydrate moiety. This modification renders tumor gp-a incapable of combining with HCG-b. (20 Refs)

41. THE TRANSFORMING PROTEINS OF PRCII VIRUS AND ROUS SARCOMA VIRUS FORM A COMPLEX WITH THE SAME TWO CELLULAR PHOSPHOPROTEINS.

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J Virol; 43(2):448-455 1982

P105 and P110, the presumptive transforming proteins of PRCII avian sarcoma virus, have been found to present in transformed chicken cells in two forms: as monomers and a part of a complex which contains both a 50,000-dalton and a 90,000-dalton cellular phosphoprotein. The 90,000-dalton cellular protein was found to be identical to one of the proteins in chicken cells whose synthesis is induced by stress. The 50,000-dalton proteins was found to contain phosphotyrosine when isolated from the complex and therefore may be a substrate for the tyrosine protein kinase activity which is associated with P105 and P110. These same two cellular phosphoproteins have previously been shown to be present in a complex with pp60src, the tyrosine protein kinase which is the transforming protein of Rous sarcoma virus. However, not all avian sarcoma virus transforming proteins with associated tyrosine protein kinase activities form a complex efficiently with these cellular proteins. Little if any of P90, the putative transforming protein of Yamaguchi 73 virus, was found in a complex with the 50,000-dalton and 90,000-dalton cellular phosphoproteins. (Author abstract) (34 Refs)

42. INTERACTION OF ALPHA,BETA-DIETHYL STILBESTROL 4,4'-BISPHOSPHATE WITH ARGINYL SUBSTRATES RESULTING IN APPARENT INHIBITION OF TRYPSIN AND THROMBIN.

Steven FS, Griffin MM, Hulley TP, Brooman P
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Eur J Biochem; 125(2):305-309 1982

Alpha,beta-diethyl stilbestrol 4,4'-bisphosphate (up to 7.0 mM) had no inhibitory action on trypsin assayed with the lysyl substrate, L-lysine nitroanilide dihydrobromide or by active-site titration with 4-methyl umbelliferyl-p-guanidinobenzoate-HCl. Diethyl stilbestrol bisphosphate had an inhibitory action on the tryptic cleavage of arginyl peptides when fluorescein-labeled soluble casein was used as a substrate. This inhibitory action was caused by complex formation between diethyl stilbestrol bisphosphate and susceptible arginyl residues in the substrate, inhibition being reversed by increasing the substrate concentration or adding competitive arginine molecules. This type of inhibition involving substrate modification is referred to as 'apparent inhibition' of trypsin and was confined to arginyl peptide bond cleavage. Thrombin specifically cleaves arginyl bonds in fibrinogen to initiate clot formation by the production of fibrin monomers. Diethyl stilbestrol bisphosphate was shown to cause apparent inhibition of clot formation in whole plasma and fibrinogen solutions in the presence of added thrombin. Kinetic data of clot formation demonstrated a threshold level of diethyl stilbestrol bisphosphate (approx 5 mM) necessary to be exceeded before these apparent inhibitory effects were observed. The mechanism of 'apparent inhibition' is briefly discussed in relation to the more usual inhibition of an enzyme activity due to the direct action of an inhibitor with the enzyme under study. (Author abstract) (9 Refs)

BINDING/ RECEPTOR PROTEINS

43. BINDING OF BENZO(A)PYRENE TO HEPATIC CYTOSOLIC PROTEIN ENHANCES ITS MICROSOMAL OXIDATION.

Dixit R, Bickers DR, Mukhtar H
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Biochem Biophys Res Commun; 104(3):1093-1101 1982

A study with 14C-benzo(a)pyrene (BP) indicates that its binding to a protein in rat liver cytosol facilitates its oxidation by microsomal enzymes. Cytosols (100,000 x gravity supernatants) were prepared from the liver of Sprague-Dawley rats. The cytosols saturated with BP were subjected to Sephadex G-100 gel permeation chromatography. Analysis of the fractions resulted in two peaks of protein bound radioactivity. Glutathione-S-transferase activity (towards 1-chlor 2,4-dinitrobenzene as substrate) eluted as a single major peak which coincided with one peak of protein bound BP. Oxidation of cytosolic protein bound BP (GST rich fractions) by microsomes from control or 3-methylcholanthrene-treated rats was significantly enhanced as compared to ethanol suspended BP. Production of oxidized products from the protein-bound BP was dependent on incubation time and microsomal protein concentration, it required NADPH and was inhibited by monooxygenase inhibitors (alpha-naphthoflavone, 1-benzylimidazole, metyrapone, and SKF 525A). Coemergence of BP binding-protein with GST suggests that the soluble protein could be one of the glutathione-S-transferases. (15 Refs)

44. THE ROLE OF CARRIER PROTEINS IN THE OXIDATION OF BENZO(A)PYRENE IN RAT LIVER (MEETING ABSTRACT).

Hanson-Painton O, Griffin MJ, Tang JN
Oklahoma Medical Research Foundation and University of Oklahoma Health, Sciences Center Oklahoma City, OK, 73104
Fed Proc; 41(3):927 1982

Cytosolic proteins possess the ability to serve as carrier between particulate benzo(a)pyrene (BP) and the microsomal cytochrome P450. The carrier mechanism of the partially purified 46,000-dalton protein (C) was investigated. Using [14C] BP and Sephadex G-100 chromatography, we have demonstrated that (1) the transfer of BP from C to microsomes (M) is predominately in the forward direction, (2) C is capable of recycling between BP and M, (3) BP in isolated BP-M can be oxidized by the addition of NADPH, (4) the oxidized BP (BP*) in M can then be transferred to fresh C in the forward direction, and (5) BP* in M will not dissociate from M without the presence of C. The presence of BP and BP* in various fractions has been shown by HPLC (high pressure liquid chromatography). Thus C can be a carrier for both BP and BP*. The direction of transfer is the same as expected for metabolism of BP in vivo, suggesting that C has such an in vivo role. (no Refs)

45. IDENTIFICATION OF CA++-CALMODULIN DEPENDENT ENZYME ACTIVITIES IN AN INSULIN-SECRETING TUMOUR.

Penn EJ, Hutton JC, Hales CN
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Biochem Soc Trans; 10(4):266-267 1982

The nature of the major calmodulin-binding proteins was investigated in a transplantable rat islet-cell tumor. The eluted proteins, obtained by affinity chromatography from post-microsomal supernatant, representing 1% of the total tissue protein, were distributed among a large number of different peptides. The association between calmodulin and tumor proteins could be reversed by trifluoperazine and required Ca++ (but not Mg++). Investigation of the eluted protein fraction indicated the presence of myosin light-chain kinase, cyclic nucleotide phosphodiesterase, and phosphorylase kinase. Further investigation of myosin light-chain kinase activity indicated a relationship to enzyme from other previously described non-muscle and smooth muscle cells. The observation that insulin-secretory tissue contains myosin light-chain kinase activity, taken together with the previously reported presence of actin and myosin, is consistent with the involvement of Ca++-regulated contractile processes in the secretory mechanism. (19 Refs)

46. IDENTIFICATION OF A GLUCOCORTICOID RECEPTOR IN THE HUMAN LEUKEMIA CELL LINE K562.

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(c/o Gidari) Dept. Pharmacology, State Univ. New York, Downstate Medical Center, 450 Clarkson Ave., Box 29. Brooklyn, NY, 11203
J Lab Clin Med; 100(2):178-185 1982

The binding of [3H]dexamethasone to the cytosol fraction prepared from the human leukemia cell line K562 was studied with a competitive binding assay. Specific, saturable binding was identified by incubating cytosol with increasing concentrations of [3H]dexamethasone in the presence and absence of nonlabeled dexamethasone. A Scatchard plot of the data was linear, suggesting the presence of a single class of binding sites with KD = 2.49 +-0.23 x 10(-8) M. the binding sites appear to be protein in nature, since specific binding was reduced by treatment of the cytosol with trypsin, pronase, and heat; neither DNase nor RNase affected the binding. Binding was also reduced in the absence of alpha-thioglycerol and in the presence of p-chloromercuribenzoate and N-ethylmaleimide, suggesting that optimal binding activity requires reduced sulfhydryl groups. The binding site appears to be specific for glucocorticoids as evaluated in competition studies. Finally, glucocorticoids were found to inhibit the clonal growth of K562 cells in vitro, suggesting a potential role for glucocorticoid binding sites in the modulation of K562 cell proliferation. (Author abstract) (33 Refs)

47. CHARACTERIZATION OF FC RECEPTORS FOR IGE ON HUMAN ALVEOLAR MACROPHAGES.

Melewicz FM, Kline LE, Cohen AB, Spiegelberg HL
(c/o Spiegelberg), Scripps Clinic and Res. Foundation, 10666 N. Torrey Pines Road, La Jolla, CA, 92037
Clin Exp Immunol; 49(2):364-370 1982

Human alveolar macrophages (aMphi) isolated from lung lavages performed during bronchoscopy and after surgical removal of pulmonary lobes were analyzed for Fc receptors for IgE (Fc epsilon R) and IgG (Fc gamma R) by rosette assays. A mean +-1 s.d. of 8.0 +-2.6% of aMphi formed rosettes with fixed ox erythrocytes coated with an IgE myeloma protein (Eo'-IgE). The Eo'-IgE rosettes were inhibited by two IgE myeloma proteins and by IgE Fc fragments but not by myeloma proteins of the other Ig classes or by IgE denatured by heating or reduction and alkylation. Fresh ox erythrocytes sensitized with rabbit IgG antibodies (EoA) formed rosettes with 64.1 +-20.3% of the aMphi. Peripheral blood monocytes formed 10.6 +-1.2% Eo'-IgE and 90.2 +-1 6.0% EoA rosettes. Incubation of the aMphi with a goat antiserum to human lymphocyte Fc epsilon R inhibited Eo'-IgE rosette formation on aMphi by 80% but did not affect the percentage of EoA rosettes. The antiserum also inhibited Eo'-IgE rosettes formed by peripheral blood monocytes and cultured macrophage-like U937 cells but not those formed by basophilic granulocytes obtained from a patient with chronic myelogenous leukemia. There was no relationship between age, sex, diagnosis or smoking history of the patients and the percentage of aMphi forming Eo'-IgE rosettes. These studies demonstrate that a subpopulation of human aMphi bear Fc epsilon R that share antigenic determinants with Fc epsilon R on lymphocytes and monocytes. Fc epsilon R(+) aMphi may play an important role in allergic and inflammatory pulmonary diseases by inducing the release of mediators of inflammation after interaction with IgE immune complexes. (Author abstract) (24 Refs)

48. INTERACTIONS OF C-REACTIVE PROTEIN WITH LYMPHOID CELLS.

James K, Baum LL, Vetter ML, Gewurz H
Dept. Immunology/Microbiology, Rush Medical Coll., Chicago, IL, 60612
Ann NY Acad Sci; 389:274-285 1982

The interactions between CRP and peripheral blood lymphocytes were investigated. CRP, in the presence of an appropriate ligand, bound saturably to a small percentage of normal PBL. The characteristics and optimal conditions for this binding were defined using several different assay systems. CRP was found to bind preferentially to cells with the IgG FcR. Binding was increased in the presence of acute phase sera, and higher numbers of cells binding CRP were observed in acute phase individuals. CRP and CRP-CPS had minimal effects upon lymphocyte responsiveness in vitro, although enhancing effects on MLC and CMC reactions, and a slight blastogenic effect, were observed. CRP antigenicity was detected on a small percentage of PBL, and treatment of PBL with anti-CRP and complement led to loss of NK reactivity, suggesting a possible association of CRP with this function. The functional expression of binding of CRP complexes, the relationship of the CRP-binding site to surface CRP antigenicity and the FcR, and the role of these factors in lymphocyte functions such as NK reactivity and recognition, are yet to be determined. (Author abstract) (26 Refs)

49. SOLUBILIZATION AND PURIFICATION OF THE PHORBOL ESTER TUMOR PROMOTER RECEPTOR FROM MOUSE BRAIN (MEETING ABSTRACT).

Ashendel CL, Boutwell RK
McArdle Laboratory, University of Wisconsin, Madison, WI, 53706
Fed Proc; 41(3):492 1982

In order to understand the molecular mechanism of action of the potent mouse skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) and its interaction with the membrane bound TPA receptor, it was necessary to purify this protein from mouse brain, the tissue with the highest specific binding activity (25 pmol/mg particulate protein). Solubilization from the crude particulate preparation required detergent; 10 mg/ml solutions of mild detergents such as sodium cholate, 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), and Triton X-100 gave the highest yields of solubilized activity. Purification was accomplished by DEAE (diethylaminoethyl) cellulose column chromatography, gel filtration chromatography using Sephacryl S-300 and S-200, and hydroxylapatite column chromatography. All chromatography was done using buffers containing CHAPS at a concentration of 2.5 mg/ml. The specific binding activity of the purified protein was at least 50 times greater than the crude particulate protein. In order to assay the TPA binding by the cold acetone filtration method it was necessary to reconstitute the solubilized binding protein by dilution in the presence of soybean lipid and $MgCl_2$. Purified, reconstituted protein had the same affinity for TPA as the crude particulate protein. Further characterization of the purified TPA receptor including preparation of antibody is being persued. (no Refs)

50. SPECIFIC HIGH AFFINITY RECEPTORS FOR BIOLOGICALLY ACTIVE PHORBOL AND INGENOL ESTERS (MEETING ABSTRACT).

Shoyab M, Todaro GJ
National Institute of Health, National Cancer Institute, Frederick, MD, 21701
Fed Proc; 41(3):337 1982

A variety of normal and transformed avian and mammalian cells and tissues contain high affinity specific receptors which interact with biologically active phorbol and ingenol esters in a reversible and saturable manner. The binding of labelled phorbol-12,13-dibutyrate (PDBu) to live or glutaraldehyde-fixed cells is dose-, time- and temperature-dependent. Those phorbol or ingenol esters which stimulate cell growth in culture and have tumor-promoting activity in vivo inhibit the binding of labelled PDBu, while the biologically inactive derivatives fail to do so. Other non-diterpene tumor-promoting agents, epidermal growth factor, retinoids and prostaglandins do not compete for the binding of labelled PDBu to its specific membrane receptors. PDBu binding sites are widely distributed in mouse tissues. Brain and spleens contain exceptionally high concentrations of these receptors. PDBu receptors are present in all regions of the brain. The choroid plexus and pituitary bind markedly lower amounts of PDBu than other brain regions. Brains or spleens from different murine strains bind almost the same amounts of PDBu while those organs from embryonic mice bind very little PDBu. Binding increases with age and reaches a maximum at about 4 weeks of age. Young and mature brains from various species contain these specific binding sites. These data suggest a structural or enzymatic role for PDBu receptors. (no Refs)

51. IMMUNOLOGICAL SUBTYPING OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) USING PEANUT LECTIN (ARACHUS HYPOGEA) (MEETING ABSTRACT).

Coffey T, Russell EC, Mohanakumar T
Medical College of Virginia, Richmond, VA, 23298
Fed Proc; 41(3):727 1982

Childhood ALL is composed of subgroups defined by immunologic markers and certain markers are known to have prognostic significance. Null cell ALL is the largest subgroup and additional markers are needed to identify patients at increased risk of early relapse within this non-T, non-B category. We have used a direct immunofluorescence assay to identify the presence of receptors for the lectin peanut agglutinin (PNA) on blasts from 26/46 (63%) of our null cell ALL patients and have subsequently noted a high rate of relapse (17/26) in the PNA+ group. Relapse has occurred in only 5/20 patients whose blasts lacked the PNA receptor. The PNA+ and PNA- groups were comparable in clinical features (ie, age, leukocyte count, follow-up times) and the striking difference in relapse rate was correlated only with the presence of receptors for the lectin. PNA receptors are commonly found only on fetal thymocytes but not on normal mature peripheral lymphocytes. SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) analysis of NaB3H4 galactose oxidase labelled solubilized cell membrane extract revealed a 68,000 dalton glycoprotein found only on PNA+ ALL lymphoblasts. Neither PNA- blasts nor PNA+ normal thymocytes expressed this component. The lectin PNA seems to define a high risk group of null cell ALL patients and to be associated with a specific cell membrane component. (no Refs)

52. TRANSFERRIN RECEPTOR EXPRESSION AND GROWTH OF LEUKEMIA CELL LINES (MEETING ABSTRACT).

Neckers LM, Cossman J
Laboratory of Pathology, National Cancer Institute, Bethesda, MD, 20205
Fed Proc; 41(3):740 1982

Most human malignant cell lines require transferrin for cell growth. Using a monoclonal antibody, OKT9, that recognizes the receptor for transferrin we have examined the expression of this receptor during cell growth. All 4 T-cell acute lymphocytic leukemia cell lines tested (Molt-4, CEM, 8402, and HSB) were OKT-9-positive by fluorescence activated cell sorter analysis. A single addition of OKT9 to all cell lines completely inhibited their proliferation for three days without affecting viability. The fluorescence intensity (receptor density) positively correlated with the concentration of cells during exponential growth. In a simultaneous analysis of cell cycle phase and OKT9 reactivity, OKT9-positive cells were present in all phases of the cell cycle and their percentage was maximal (77%) in G2+M. Addition of methotrexate to growing cells reduced the percentage of OKT9-positive cells from 74% to 37%. Deletion of non-essential amino acids from the culture media simultaneously resulted in reduced cell growth (30% of control) and a decrease in fluorescence intensity of OKT9-stained cells (54% of control). A second monoclonal antibody, Leu 1, reacted with all 4 cell lines but did not vary with cell concentration, addition of methotrexate or deletion of non-essential amino acids. Unlike OKT9, addition of Leu 1 to growing cells had no effect on their growth. (no Refs)

53. A NOVEL SINGLE-STRANDED DNA-BINDING PROTEIN, FROM THE NOVIKOFF HEPATOMA, WHICH STIMULATES DNA POLYMERASE (MEETING ABSTRACT).

Koerner TJ, Meyer RR
University of Cincinnati, Cincinnati, OH, 45221
Fed Proc; 41(3):1196 1982

Single-stranded DNA-binding proteins (SSB) are thought to play important roles in DNA replication and repair. We have isolated and purified to homogeneity, a novel SSB from the rat Novikoff ascites tumor which has properties distinct from other mammalian SSBs. The protein binds cooperatively, but weakly, to single-strand DNA; it is capable of denaturing duplex DNA as determined by nuclease S1 sensitivity and by electron microscopy, and will catalyze helix destabilization of poly(dA-dT) (poly(deoxyadenylate-thymidylate)) at very low temperatures. The protein is a single polypeptide of Mr 48,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, gel filtration and glycerol gradient sedimentation. The amino acids composition differs from any other eukaryotic SSB so far described, but like these other binding proteins, the Novikoff hepatoma SSB is not high in basic amino acids. This SSB stimulates the activity of DNA polymerase, and this stimulation appears to be enhanced further by the presence of a DNA-dependent ATPase. The Novikoff hepatoma SSB thus plays a role as an accessory protein for DNA synthesis in vitro. (no Refs)

54. SPECIFIC BINDING OF 1,25-DIHYDROXYCHOLECALCIFEROL IN HUMAN MEDULLARY THYROID CARCINOMA.

Freake HC, MacIntyre I
Endocrine Unit., Royal Postgraduate Medical Sch., Du Cane Road, London W12 0HS, England
Biochem J; 206(1):181-184 1982

A specific 1,25-dihydroxycholecalciferol-binding protein has been detected in high-salt cytosols prepared from human medullary thyroid carcinomas. The binding protein had the same equilibrium dissociation constant (Kd = 0.17 +-0.05 nM; n = 4) and sedimentation coefficient on sucrose gradients (3.7S) as that seen in established vitamin D target tissues. This protein was not detected in normal thyroid cytosols, which may reflect the low proportion of C-cells within the gland. (Author abstract) (22 Refs)

MEMBRANE/ SURFACE PROTEINS

55. A UNIQUE CELL-SURFACE PROTEIN PHENOTYPE DISTINGUISHES HUMAN SMALL-CELL FROM NON-SMALL-CELL LUNG CANCER.

Baylin SB, Gazdar AF, Minna JD, Bernal SD, Shaper JH
Oncology Center, Dept. Medicine, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
Proc Natl Acad Sci USA; 79(15):4650-4654 1982

We have used radioiodination (125I) and two-dimensional polyacrylamide gel electrophoresis to determine that small-(oat) cell lung carcinoma (SCC) - a tumor with neuroendocrine features - possesses a surface protein pattern distinct from the other types of lung cancer cells (squamous, adeno-, and large-cell undifferentiated carcinoma). Twelve distinguishing proteins, 40 to 70 kilodaltons (kDal), characterized four separate lines of SCC; three of these, designated E (60 kDal; pI = 7.3), S (30 kDal; pI = 6.0), and U (57 KDal; pI = 5.6), may be unique SCC gene products and were identified only in [35S]methionine labeling of SCC and not in non-SCC or human fibroblasts. Two lines of adeno-, one of squamous, and one of undifferentiated large-cell lung carcinoma exhibited similar surface protein patterns to one another. Nine distinguishing proteins (40 to 100 kDal) and at least five large proteins (greater than 100 kDal) were unique to these lines. The surface protein phenotypes for SCC and non-SCC were distinct from those for human lymphoblastoid cells and fibroblasts. However, the neuroendocrine features of SCC were further substantiated because 6 of the 12 distinguishing SCC surface proteins, including E and U, were identified on human neuroblastoma cells. The proteins identified should (i) help define differentiation steps for normal and neoplastic bronchial epithelial cells, (ii) prove useful in better classifying lung cancers, and (iii) be instrumental in tracing formation of neuroendocrine cells. (Author abstract) (34 Refs)

56. TWO-DIMENSIONAL ELECTROPHORESIS PATTERNS OF MEMBRANE PROTEINS FROM LEUKAEMIC MYELOBLASTS AND NORMAL GRANULOCYTES AFTER VARIOUS ECTOLABELLING PROCEDURES (MEETING ABSTRACT).

DeJong JG, Dekker AW, Kapteijn R, Sixma JJ
University Hospital, Utrecht, The Netherlands
Br J Haematol; 50(4):704 1982

In order to study membrane proteins of myeloid leukemia cells and normal granulocytes we labelled cells by the following ectolabelling procedures: (1) lactoperoxidase mediated iodination; (2) labelling of sialic acid residues by mild periodate oxidation and [3H]borohydride reduction; (3) labelling of terminal galactose residues with galactose oxidase and [3H]borohydride after neuraminidase treatment; (4) metabolic labelling with [3H]fucose. Membrane protein composition was then analyzed by two-dimensional electrophoresis (isoelectric focusing and sodium dodecyl sulphate electrophoresis). Radiolabelled proteins were detected by autoradiography or fluorography. After 125I/lactoperoxidase labelling both in granulocyte and myeloblast patterns 20-25 different labelled proteins could be indicated. Resolution was much better than after one-dimensional electrophoresis. Patterns after 125I/lactoperoxidase and [3H]borohydride labelling were in agreement with each other in that most of the proteins labelled by the lactoperoxidase method were also labelled by the borohydride procedure although there were differences in intensity. [3H]fucose labelled cells gave spots much more condensed than the apparently more heterogenously labelled proteins after the other labelling procedures. It was difficult to find correspondence with the patterns after 125I/lactoperoxidase or [3H]borohydride procedures. After 125I/lactoperoxidase and [3H]borohydride labelling most of the label was in the acid region of the gel both for granulocytes and leukemic myeloblasts. Major differences between leukemic myeloblasts and granulocytes were in the molecular weight region of 120 kd pI 5 and in the more neutral pI region at molecular weights below 45 kdaltons. (no Refs)

57. TWO-DIMENSIONAL ELECTROPHORESIS (2-DE) OF CELL PROTEINS IN CHILDHOOD LEUKEMIA (MEETING ABSTRACT).

Hanash SM, Tubergen DG, Heyn RM, Neel JV, Sandy L, Stevens GS, Rosenblum BB, Springstead KN
University of Michigan Medical School, Ann Arbor, MI
Pediatr Res; 16(4,part2):205A 1982

We have investigated the potential of 2-DE to identify differences in the protein patterns of various types of childhood leukemia. Solubilized proteins in normal lymphocytes and in blast cells from children with acute lymphoblastic (ALL) or myelogenous leukemia (AML) were electrophoresed using Andersons' ISODALT system. Protein spots were visualized with Coomassie staining followed by a modification of Merrill's silver staining technique. Approximately 1000 spots were routinely visualized in individual gels. Various spots gave characteristic shades of color from blue to yellow red and brown. Identical patterns were observed in multiple runs of the same sample. Initial Analysis included the 400 major spots in the gels. Analysis was facilitated by cytocomputer imaging of specific constellations of spots. A total of five spots were present in all five AML samples and consistently non-detectable in ten ALL samples including seven non-T, non-B and three T-cell ALL. Four spots were present in ALL but not in AML samples. Five spots were present in ALL samples but not in normal lymphocytes. (no Refs)

58. CELL SURFACE INTERACTIONS OF NORMAL AND VARIANT H6 TERATOCARCINOMA CELLS (MEETING ABSTRACT).

Kartha S, Felix JS
Department of Pediatrics, Johns Hopkins University, School of Medicine, Baltimore, MD
Pediatr Res; 16(4,part2):113A 1982

Mouse teratocarcinoma cell cultures share many characteristics with the early mouse embryo and are a suitable model for studying aggregation and compaction during preimplantation development. H6 cells form uniform, grape-like clusters of adhering cells when rotated in medium with 10% fetal calf serum. These aggregates are induced to compact within 1 hour when additional calcium (5mM) is supplied, resulting in a tight mass of cells with obscure cell boundaries and a smooth outer surface. Compaction can be reversed by removal of calcium and inhibited by cytoskeletal poisons and by the lectins concanavalin A and wheat germ agglutinin. This behavior is similar to compaction in the 8 cell morula. Mutants of H6 with altered aggregation or compaction will be useful for studying the molecular nature of these processes. We have isolated 3 aggregation-deficient variants which have lost the ability to form intercellular adhesions. Since cell surface glycoproteins are implicated in cell adhesions, agglutination by eight lectins was tested. Only peanut agglutinin (PHA) had a significantly reduced effect on all 3 variants compared to parental cells. Measurement of fluoresceinated-PNA bound to the cell surface confirmed this deficiency; the mean fluorescence value for parental cells was reduced to 1/3 in the variant lines, suggesting that a galatose-rich glycoprotein is defective or missing in the variants and is associated with aggregation. (no Refs)

59. DNA-BINDING DOMAINS OF HUMAN FIBRONECTIN.

McMaster GK, Zardi L
(c/o Zardi), Istituto Scientifico per lo Studio e la Cura dei Tumori di Genova, Viale Benedetto XV, 10, I-16132 Genova, Italy
Biochem Biophys Res Commun; 107(2):609-617 1982

DNA-binding domains of human plasma fibronectin were studied by the bidirectional 'protein blotting' technique. This technique has the advantage that minute quantities of tryptic peptides can be directly analyzed for their interaction with macromolecules after blotting on nitrocellulose filters. The fibronectin was isolated by affinity chromatography and its tryptic digest was separated on a sodium dodecylsulfate-polyacrylamide gel. The polypeptides were transferred to nitrocellulose sheets and the DNA-binding peptides were identified by incubation of the nitrocellulose sheets with 32P-labeled human DNA followed by autoradiography. Only a few polypeptides of the tryptic digest interacted with DNA. The major DNA binding fragments were 150, 125, 110, 85, 60 and 50 kilodaltons (kd). The 50 kd and 60 kd polypeptides, which were not predominant bands as visualized by amido black or Coomassie blue staining, bound much more labeled human DNA than other polypeptides present in larger amounts. The heavily stained 35 kd and 55 kd bands did not bind DNA. In a study of the specificity of the interaction between DNA and fibronectin, a 300-fold excess of Escherichia coli DNA did not inhibit the human DNA-human fibronectin binding, indicating the specificity of the interaction. (26 Refs)

60. COMPARATIVE IMMUNOHISTOCHEMICAL LOCALIZATION OF FIBRONECTIN AND ACTIN IN HUMAN BREAST TUMOR CELLS IN VIVO AND IN VITRO. I. FIBROADENOMA.

Gilbert MA, Noel P, Faucon M, De Ceccatty MP
Unite de Morphologie Cellulaire et Tissulaire, Centre Leon Berard, 28 Rue Laennec, F-69373 Lyon Cedex, France
Virchows Arch [Cell Pathol]; 40(1):99-112 1982

The presence and distribution of fibronectin and actin in breast fibroadenoma cells have been investigated in frozen sections and primocultures of the same samples, by means of indirect immunofluorescence techniques. In the tissue cells, epithelial cells were negative whereas myoepithelial cells were positive with the two antibodies. Moreover, fibronectin was mainly distributed in basal lamina of ducts and ductules whereas actin appeared to label stroma cells. In primocultures obtained from the same samples of fibroadenomas, the labeled patterns of fibronectin and actin were not really distinguishable between cells; the two protein distributions were quite usual. Our data on human breast fibroadenoma cells are in agreement with the concepts of cellular adaptation or selection in culture and emphasize the difficulty in correlating results of 'in vitro' and 'in vivo' studies. (Author abstract) (41 Refs)

61. FIBRONECTIN ENHANCES IN VITRO MONOCYTE-MACROPHAGE-MEDIATED TUMORICIDAL ACTIVITY.

Perri RT, Kay NE, McCarthy J, Vessella RL, Jacob HS, Furcht LT
Hematology-Oncology Section (IIIE), Veterans Admin. Medical Center, 54th St. and 48th Ave. South, Minneapolis, MN, 55417
Blood; 60(2):430-435 1982

Control of neoplastic proliferation reflects in part monocyte/macrophage destruction of target cells - destruction that evidently requires cell-cell interaction. We herein show it to involve the natural plasma opsonin, fibronectin. With two cultured human tumor lines - Malme melanoma and CAK-I renal carcinoma cells - addition of fibronectin, purified to homogeneity, enhances macrophage-mediated cytotoxicity two to four fold (p less than 0.01). Both fresh human monocytes or the U-937-cultured macrophage line become more lethal to tumor cells with added fibronectin. The fibronectin-enhanced monocyte and U-937 tumoricidal activity occurred in a dose-dependent fashion. Specificity of fibronectin's action was validated by use of affinity-purified rabbit antifibronectin antibody, which completely abated its enhancement of tumoricidal activity. Enhancement of tumoricidal activity did not occur when monocytes or U-937 were exposed to fibronectin-coated plates. However, the addition of soluble fibronectin to fibronectin-coated plates was then capable of enhancing cytocidal activity. These studies demonstrate that human fibronectin is capable of increasing both fresh and cultured human monocyte tumor-directed cytotoxicity. Fibronectin appears to be a potentially important circulating molecule that may favorably influence human monocyte tumor cell cytotoxicity. (Author abstract) (36 Refs)

62. PLASMA FIBRONECTIN IN ACUTE LEUKAEMIA.

Boughton BJ, Simpson A
Dept. Haematology, Queen Elizabeth Hosp., Birmingham B15 2TH, England
Br J Haematol; 51(3):487-491 1982

In acute leukemia, low plasma fibronectin was seen at diagnosis and when the disease relapsed. This was not directly related to tumor load, chemotherapy or neutropenia, but correlated well with episodes of intercurrent infection. In severe infections, fibronectin fell rapidly to very low levels, and was sometimes not restored to normal up to 2 wk later. Preliminary attempts at replacement therapy were successful and the possible importance of this is discussed. (Author abstract) (10 Refs)

63. HISTOCHEMICAL STUDIES ON FIBRONECTIN IN HUMAN NEOPLASMS BY IMMUNOPEROXIDASE TECHNIQUE (MEETING ABSTRACT).

Matsuda T, Yasutomi M
Department of Surgery, Kinki University, Osaka, Japan
IXth Biennial Congress of the International Society of University Colon and Rectal Surgeons, June 6-10, 1982, Munich, W. Germany. Society of University Colon and Rectal Surgeons 175 pp., 1982.

Fibronectin is a high molecular weight glycoprotein and is reported to be involved in cell adhesion or malignant transformation under cell culture condition. Systematic histochemical studies on fibronectin, however, have scarcely been undertaken so far on intestinal tumors. We, therefore, have conducted histochemical studies on fibronectin in cancer and adenoma of the large intestine by immunoperoxidase technique. 1) With regard to fixation method, immunohistochemical reaction of fibronectin in a frozen section resulted in an active one, whereas it was remarkably suppressed when the section was fixed with formalin. 2) In normal mucosa of the large intestine, a reaction of fibronectin was found active in basal membrane of epithelial cells and inactive in the stroma cells of the lamina propria mucosa. 3) In cancer cells, the reaction of fibronectin was totally absent in the basal part of the gland. 4) In the case of adenoma, there existed a correlation between the intensity of fibronectin staining and the degree of epithelial atypism, and in a severe case of atypism it appeared less active than in the case of adenocarcinoma. It was verified that this technique will serve as a useful supplementary technique for the purpose of studying the biological role of fibronectin. (no Refs)

64. VIE-G4, A MONOCONAL ANTIBODY REACTING WITH GLYCOSYLATED GLYCOPHORIN A ON HUMAN ERYTHROCYTES, ERYTHROID PRECURSORS AND K-562 CELLS, BUT NOT ON ACUTE LEUKEMIC CELLS (MEETING ABSTRACT).

Liska L, Majdic O, Bettelheim P, Knapp W
Department of Internal Medicine, University of Vienna, Vienna, Austria
Fifth European Immunology Meeting, June 1-4, 1982, Istanbul, Turkey. Turkish Society of Immunology 354 pp., 1982.

The VIE-G4 monoclonal IgM kappa type antibody raised against intact human erythrocytes reacts with all erythrocytes, the morphologically recognizable precursors of erythroid differentiation ie all reticulocytes, most of the normoblasts and some erythroblasts with less degenerated nuclei. Isolated cells from 38 acute myeloid leukemia (AML) patients and from 48 different acute lymphoid leukemia (ALL) cases showed no reactivity with VIE-G4. The one patient with morphologically recognizable erythroleukemia had 45% reactive bone marrow cells. Cells of different hemopoietic cell lines were negative, except the K-562 cells showing 40% staining in indirect immunofluorescence assay. Immunoprecipitation of surface iodinated erythrocyte ghost extracts revealed that VIE-G4 defines a membrane sialoglycoprotein with an apparent molecular weight of 41,000 daltons, representing the glycosylated form of glycophorin A, since the reactive antigenic site is eliminated upon desialization by treatment with neuraminidase. (no Refs)

65. PULMONARY CYTOCHROMES P-450 FROM RABBITS TREATED WITH 3-METHYLCHOLANTHRENE (3-MC) (MEETING ABSTRACT).

Ueng TH, Alvares AP
Department of Pharmacology, Uniformed Services University, Bethesda, MD, 20814
Fed Proc; 41(3):1295 1982

Pretreatment of rabbits with 3-MC caused a consistent increase in total cytochrome P-450 content and benzo(a)pyrene hydroxylate activity, but no change in ethylmorphine N-demethylase activity in lung microsomes. In addition, the CO difference spectrum of the reduced microsomes showed a shift in the Soret maximum. A slight increase in the ratio of 455:430 nm peaks in the ethyl isocyanide difference spectrum of pulmonary microsomes was also observed. Column chromatographic separation of pulmonary cytochromes P-450 demonstrated that 3-MC had no significant effect on the constitutive cytochromes P-450I and P-450II. However, 3-MC induced a new form of this hemeprotein, designated P-450III. The CO difference spectra of cytochromes P-450I, P-450II and P-450III showed absorption maxima at 452, 450 and 448.4 nm, respectively. In addition, the ethyl isocyanide difference spectral properties of cytochrome P-450II had a slower reduction rate and was less stable than P-450III were different from the other two isozymes. At room temperature, cytochrome P-450III had a slower reduction rate and was less stable than P-450II. SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), analysis revealed that P-450III had a slower electrophoretic mobility than P-450I and P-450II. Following electrophoresis, P-450III and P-450I showed marked decreases in heme-associated peroxidase activity, when compared to P-450II. These studies demonstrate that 3-MC has the ability to induce a new and distinct form of cytochrome P-450 in rabbi lung. (no Refs)

66. CHEMOTACTIC PROPERTIES AND BIOCHEMICAL FEATURES OF MALIGNANT CELLS IN VITRO (MEETING ABSTRACT).

Mensing H, Albini A, Krieg T, Muller P, Pontz B, Meigel W
University Hautklinik, 2000 Hamburg, W. Germany
J Invest Dermatol; 78(4):331 1982

The pathophysiology of expansive growth and metastasis of malignant tumors is little understood. One of the crucial features of tumor-cells is their motility and response to chemoattractants like fibronectin (FN) and collagen derived peptides (CDP). Since tumor-cells are known to have an altered synthesis of collagen and FN resulting in a reduced connective tissue matrix it was the aim of this study to investigate their chemotactic response to FN and CDP. Rhabdomyosarcoma-, fibrosarcoma-, SV 40 transformed human lung fibroblasts and 5 cell strains derived from human dermatofibrosarcoma protuberans - each characterized by the synthesis of a specific pattern of structural proteins - were grown and compared to normal human fibroblasts in respect to their synthesis of collagen, FN and their chemotactic activity. In result chemotaxis was correlated to the amounts of FN synthesis of the different cell types: Cells, producing less FN show markedly increased chemotactic migration, when FN or CDP are used as attractant. In addition less minimal concentration of attractant was necessary to stimulate chemotaxis in these cells. In contrast no alteration of chemotactic activity could be noticed in DFP cells, which show a collagen- and FN synthesis similar to that of controls. These data suggest that idea that increased chemotactic response to relatively higher levels of FN and CDP in the environment of malignant tumors play an important role in the initial metastasizing process. In addition this could also explain the predominant location of metastases in connective tissue rich organs. (no Refs)

67. THREE ADENYLATE CYCLASE PHENOTYPES IN S49 LYMPHOMA CELLS PRODUCED BY MUTATIONS OF ONE GENE.

Bourne HR, Beiderman B, Steinberg F, Brothers VM
Div. Clinical Pharmacology, Dept. Medicine, Univ. California Sch. Medicine, San Francisco, CA, 94143
Mol Pharmacol; 22(1):204-210 1982

The nature of the phenotypic lesions affecting the guanine nucleotide-binding regulatory component (N protein) of adenylate cyclase in three mutant lines of S49 mouse lymphoma cells were studied. The mutant lines were cyc-, in which the N protein appears functionally absent; unc, in which the N protein's interactions with hormone receptors are impaired; and H21a, in which N appears to be unable to interact with the catalytic unit of adenylate cyclase. Gel filtration showed that the N protein of H21a probably does not differ in size from that of the wild-type. H21a X unc hybrid cells accumulated cyclic AMP in response to cholera toxin, but not isoproterenol or prostaglandin E1, and adenylate cyclase in membranes of the hybrid was phenotypically unc, indicating that it is highly likely that the defects in both mutants are caused by defects in the same gene. Similarly, both the H21a X cyc- and unc X cyc- hybrids failed genetic complementation, indicating that the three parental types bear mutations of the same gene. Results of agonist binding to beta-adrenergic receptors supported the complementation experiments. All three mutations are probably in a structural gene that codes for both the 42,000 and 52,000-dalton subunits of the N protein; this interpretation fits biochemical and genetic evidence from this and earlier studies. (25 Refs)

68. STIMULATION OF B16 MELANOMA ADENYLATE CYCLASE ACTIVITY BY MSH RELATED PEPTIDES (MEETING ABSTRACT).

MacNeil S
Department of Human Metabolism and Clinical Biochemistry, Medical School, University of Sheffield, Sheffield, England
Br J Dermatol; 106(1):115 1982

There is now evidence that the melanosome dispersing activity of MSH depends on two 'message' sequences, corresponding to positions 5-9 (Glu-His-Phe-Arg-Trp) and 10-13 (Gly-Lys-Pro-Val NH(2)) of the molecule. As well as this rapid action on amphibian melanophores, MSH and related peptides stimulate melanogenesis both in amphibian skin and in mammalian melanocytes and melanoma cells through stimulation of tyrosinase activity. This action is known to be mediated through cyclic AMP dependent mechanisms. The ability of alpha-MSH, beta-MSH and related ACTH peptides to stimulate an adenylate cyclase enzyme in a highly pigmented B16 melanoma cell line has recently been investigated in our laboratory. The peptides alpha-MSH, beta-MSH 1-39, ACTH 1-24 all stimulated the enzyme to a similar maximal extent and with similar potency, although ACTH 1-39 was slightly less potent. The heptapeptide sequence ACTH 4-10 was, however, only a partial agonist of this enzyme as was an 11 amino acid synthetic MSH preparation. Our data suggest that at least part of the heptapeptide sequence ACTH 4-10 is necessary for the activation of the receptor-linked adenylate cyclase system but that this sequence alone does not contain all of the structural features required for full activation of the enzyme. It could well be that melanoma cells recognize the same two 'message' sequences on the MSH peptide that determine the melanosome dispersing activity and it is possible that both sequences are required for full activation of adenylate cyclase and hence melanogenesis. However, while the sequence ACTH 14-24 reduces the melanosome dispersing action of ACTH 1-13 (alpha-MSH), in the frog melanophore, in our studies using the B16 melanoma, the adenylate cyclase response to ACTH 1-13 was reduced by the sequence ACTH 25-39. Furthermore, melanotrophic potentiating factor (MPF), which by itself failed to activate the B16 adenylate cyclase receptor, had no effect on the response to alpha-MSH whereas it has been reported to potentiate the actions of alpha-MSH in lizard skin and the melanogenic response to alpha-MSH in hamster follicles. (6 Refs)

69. FURTHER STUDIES ON THE RELATIONSHIP BETWEEN ALLOTRANSPLANTABILITY AND THE PRESENCE OF THE CELL SURFACE GLYCOPROTEIN EPIGLYCANIN IN THE TA3-MM MOUSE MAMMARY CARCINOMA ASCITES CELL.

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JNCI; 68(6):981-988 1982

The loss of strain specificity in the TA-St mammary carcinoma ascites cell during passage in ascites form in diseased syngeneic strain A mice was confirmed by the observation that TA3-MM/2 ascites cells were capable of progressive growth in seven foreign mouse strains. Support for the hypothesis that allotransplantability in the TA3-MM lines may be due to masking of cell surface histocompatibility H-2 7a 1 antigens by large glycoprotein (epiglycanin) molecules was obtained from the finding that the capacity of the TA3-MM/2 ascites cell to absorb anti-H-2 7a 1 antibody was several times less than that of the parent TA3-St ascites cell, although it was greater than the capacity of the non-strain-specific TA3-Ha line. Further support for the location of epiglycanin molecules at the cell surface was obtained by transmission electron-microscopic observation of a high concentration of filamentous structures, usually 20-40 nm long, but often extending to 200-300 nm length at the TA3-MM/2 cell surface. Similar structures were also observed at the TA3-Ha cell surface but were not observed at the surface of the TA3-St ascites cell, a cell known to contain no detectable epiglycanin. Epiglycanin molecules, obtained by two different methods from TA3-MM/1, TA3-MM/2, and TA3-Ha ascites cells, were shown to be similar with respect to their capacities to inhibit the binding of 125I-labeled epiglycanin to its antibody, induced in the rabbit by TA3-Ha ascites cells in a radioimmunoassay. (Author abstract) (21 Refs)

70. NONCYTOLYTIC EXTRACTION OF MURINE TUMOR-SPECIFIC TRANSPLANTATION ANTIGENS WITH THE NONIONIC DETERGENT OCTYL-BETA-D-GLUCOPYRANOSIDE.

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JNCI; 69(1):131-136 1982

The nonionic detergent octyl-beta-D-glucopyranoside (C3Glu) was used to extract immunogenic tumor-specific transplantation antigen (TSTA) from intact cells and purified plasma membranes of the 3-methylcholanthrene-induced fibrosarcoma MCA-F. Pretreatment of syngeneic C3H/HeJ mice with 100 ug of C8Glu extracts induced specific immunoprotection such that mice resisted the outgrowth of MCA-F but not the antigenically distinct tumors MCA-D or MCA-2A. Incubation of intact cells with 7 mM (0.2%) C8Glu for 30 minutes at 23 C was judged to be noncytolytic because extracted cells excluded trypan blue. Preparative isoelectric focusing partially purified the MCA-F-specific antigen from crude C8Glu extracts into the pH 6.5-6.8 region of the gradient. Electrofocusing yielded 60% of the applied antigen activity and a fourfold to fivefold increase in specific activity. In addition, immunoprospective activity was obtained in 2% C8Glu extract of MCA-F plasma membranes, confirming the membrane localization of the MCA-F TSTA. Three properties of C8Glu rendered it an attractive agent for the preparation of cell surface proteins: a nonionic character, large critical micellar concentration, and its capacity to extract antigens without complete membrane solubilization or cell disruption. (Author abstract) (30 Refs)

71. SYNTHESIS OF THE BRANCHED FORM OF ERYTHROGLYCAN BY FRIEND GM979 ERYTHROLEUKEMIC CELLS.

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J Biol Chem; 257(14):8272-8276 1982

Mouse GM979 erythroleukemic cells were found to synthesize the branched form of erythroglycan, a large polylactosamine structure known to be attached to band 3 and possibly to other proteins on human erythrocytes. Total protein-derived oligosaccharides from GM979 cells, labeled metabolically with [3H]glucosamine, [3H]mannose, or [3H]galactose, were prepared by hydrazinolysis after extracting the lipids. The 3H-labeled oligosaccharides were fractionated on Sephadex G-50 revealing 10-25% of each labeled product as a high molecular weight fraction. Digestion of this [3H]glucosamine fraction with endo-beta-galactosidase from Escherichia freundii, specific for the repeating structure of Galbeta(1 to 4)GlcNAcbeta(1 to 3), resulted in the following four products upon Bio-Gel P-2 Gel filtration: (1) a disaccharide with the structure GlcNAcbeta(1 to 3)Gal, (2) a trisaccharide with the structure Galbeta(1 to 4)GlcNAcbeta(1 to 3)betaGal, (3) a tetrasaccharide with the sequence Fucalpha(1 to 2)Galbeta(1 to 4)GlcNAcbeta(1 to 3)Gal, and (4) a large complex fragment which contained mannose, glucosamine, galactose, and fucose (presumably the protein linkage region). Methylation linkage analysis of the large complex fraction shows mainly the presence of 4-substituted and terminal N-acetylglucosamine; 3,6-substituted, 6-substituted, 2-substituted 2,3-substituted galactose. The GM979 cell erythroglycan is only 30% susceptible to endo-beta-galactosidase degradation probably because of the branched galactose residues, whereas the linear form of erythroglycan from human K562 cells is 60% susceptible. The branched residues in GM979 cell saccharides indicate that this mouse cell line bears an arborized erythroglycan-like glycopeptide similar to those found on human adult erythrocytes, and thus may be a source for the enzyme which transfers an N-acetylglucosamine residue to a 3-linked galactose to form a 3,6-disubstituted galactose. (Author abstract) (25 Refs)

72. SYNTHESIS OF A BRANCHED FORM OF ERYTHROGLYCAN (EG) BY FRIEND GM979 ERYTHROLEUKEMIC CELLS (MEETING ABSTRACT).

Kaizu T, Rush J, Turco SJ, Laine RA
University of Kentucky, Lexington, KY, 40536
Fed Proc; 41(3):664 1982

Total protein-derived oligosaccharides from GM979 cells, labeled metabolically with [3H]GlcN, [3H]Man, or [3H]Gal, were prepared by hydrazinolysis after extracting lipids. The [3H]-labeled oligosaccharides were fractionated on Sephadex G-50 as a high molecular weight fraction. Digestion of the [3H]GlcN fraction with endo-beta-galactosidase from Escherichia freundii, specific for the repeating structure of Galbeta(1-4)GlcNAcbeta(1-3), showed the following four products upon Bio-Gel P-2 filtration: 1) a branched and complex oligosaccharide which contained a mannose core, 4-substituted and terminal betaGlcNAc, and beta-Gal (3,6-, 2,3-, 6-, and 2-substituted) and fucose, 2) a disaccharide (GlcNAcbeta(1-3) Galbeta), 3) a trisaccharide (Galbeta(1-4)GlcNAcbeta(1-3)Galbeta), and 4) a tetrasaccharide (Fucalpha(1-2)Galbeta(1-4)GlcNAcbeta(1-3)Galbeta). From these data, GM979 cells carry a branched oligosaccharide, containing the Galbeta(1-4)GlcNAcbeta (1-3)Galbeta repeating unit. This mouse cell line bears an arborized erythroglycan similar to those found on human adult erythrocytes. (no Refs)

73. CHARACTERIZATION OF ANTIGENIC SITES OF HUMAN PROSTATIC ACID PHOSPHATASE.

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Ann NY Acad Sci; 390:16-26 1982

Human prostatic acid phosphatase (PAP) is antigenically uniquely different from acid phosphatases of other tissue origins. Nevertheless, a small degree of antigenic cross-reactivity between PAP and other lysosomal acid phosphatase(s) (LAP) has been suspected. In order to resolve this question, we have adopted two approaches: one involving structural studies by peptide mapping, and the other involving topological mapping through the use of uniquely defined antibodies. Purified PAP was dissociated into subunits and was further cleaved by chemical and enzymological methods. The limited digestion of PAP by submaxillary protease yielded three fragments (Sp-1, 2, and 3). One of the fragments, Sp-3 (Mr = 11,000-12,000), was shown to regain catalytic activity after interaction with anti-PAP antibodies. This along with other data suggested that the active site is localized in the Sp-3 fragment. These submaxillary protease fragments were also used in the antigenic studies. For the detailed antigenic mapping studies, we prepared 12 monoclonal anti-PAP antibodies. These monoclonal anti-PAP antibodies exhibited a remarkably specific binding to PAP, particularly to the Sp-1 fragment, without binding to other acid phosphatase preparations. We also prepared lysosomal acid phosphatase (LAP) and raised anti-LAP antibodies in rabbits. The anti-LAP antibodies were fractionated into subpopulations by the preparative isoelectric focusing method. Three anti-LAP antibody subpopulations (pI 5.2, 6.9, and 7.5) exhibited specific binding to LAP. However, two anti-LAP subpopulations (pI 5.3 and 6.8) showed binding to the Sp-3 fragment, an active site fragment of PAP. Thus, the PAP molecule seems to consist of three domains, namely, Sp-1, Sp-3, and Sp-2. Sp-3, which is the active site domain, is an antigenically cross-reactive region. The Sp-1 domain represents an antigenically unique region of PAP, whereas none of the antibodies studied thus far bind to the Sp-2 fragment. (Author abstract) (17 Refs)

74. COMPARATIVE STUDIES ON THE MAJOR COLLAGENOLYTIC ENDOPROTEINASES OF NORMAL AND MALIGNANT MURINE TISSUES.

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Diss Abstr Int [B]; 42(6):2345-B 1982

The major lysosomal sulfhydryl dependent proteinases, cathepsin B and tumor thiol proteinase were purified from acetone powders of mouse liver and a transplantable chemically induced tumor, respectively. The liver cathepsin B was determined to have a molecular weight of 30,000 and the tumor thiol proteinase, 33,000. Both enzyme preparations were mixtures of isoenzyme forms with PI's ranging from pH 4.8 to pH 5.8. Both enzymes had the same distribution of isozyme activities with the major form at pH 5.2. Both proteinases were shown to be glycoproteins and both contained 1% carbohydrate. Antiserum prepared against the tumor thiol proteinase was fully cross reactive with both native forms of the proteinases and their alkali-denaturated forms. Both enzymes exhibited marked proteolytic activity towards collagens type I and IV with a pH optimum of pH 4.2; however the liver cathepsin B more rapidly degraded collagen IV than the tumor thiol proteinase. Similarly both proteinases were capable of inactivating fructose 1,6 diphosphate aldolase, but the tumor enzyme cleaved the susceptible bonds in the substrate much more slowly than did the liver cathepsin B. The response of the two enzymes to known inhibitors of cathepsin B revealed the same pattern of inhibition of both enzymes. Ks were determined for the inhibition of the proteinases by the peptide aldehyde inhibitor, leupeptin. Comparative amino acid analysis confirmed considerable quantitative differences in amino acid compositions, suggestive of both amino acid additions and substitutions within the primary structure. Peptide fragmentation maps of the tryptic, chymotryptic and cyanogen bromide digests of the two proteinases confirmed the presence of a core sequence of high constituting 50% to 60% of the fragments while the remaining peptides were derived from non homologous sequences, suggesting different loci of genetic origin. Active site alkyaltion studies with radiolabeled reagents and peptide mapping suggests that the active site of the two proteinases have the same or similar amino acid sequence about the active site. (no Refs)

75. CATHEPSIN B-LIKE PROTEINASE IDENTIFIED AS INDUCER OF PLATELET AGGREGATION BY TUMOR CELLS (MEETING ABSTRACT).

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Fed Proc; 41(3):334 1982

Tumor cell-induced host platelet aggregation occurs in a wide variety of tumor types and is believed to facilitate tumor cell metastasis. Elutriated tumor cells from murine B16 amelanotic melanoma (B16a) induced aggregation of human washed platelets by a mechanism dependent on generation of thromboxane A2 (TxA2). We have shown that lysosomal cathepsin B activity in tumor cells correlates with metastatic potential. At low concentrations (2-5 uM) leupeptin, a cysteine proteinase inhibitor, inhibited B16a-induced platelet aggregation. CBZ-arg-arg-methoxy-beta-napthylamine, a fluorometric substrate for cathepsin B, inhibited B16a-induced but not ADP-induced platelet aggregation. Papain, a plant cysteine proteinase with an active site homologous to cathepsin B, induced platelet aggregation which was blocked by leupeptin. Papain also induced TxA2 production by platelets. Cathepsin B activity in the B16a cells was 52 uM methoxy-beta-napthylamine/mg protein x min. Cathepsin H which might also be inhibited by the substrate was not detectable in the B16a cells. Cathepsin L does not react with the substrate. The results suggest that the proteolytic enzyme responsible for tumor cell-induced platelet aggregation is a cathepsin B-like cysteine proteinase. (no Refs)

76. LEUCINE AMINOPEPTIDASE OF RAT PROSTATE CARCINOMA (MEETING ABSTRACT).

Wu CH, Blitzer A, Abramson M, Huang CC
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Fed Proc; 41(3):506 1982

Our recent work indicated that leucine aminopeptidase (LAP) and collagenase productions paralleled each other and increased with the growth of tumor in rats. LAP may be responsible for further degradation of the structural protein after the action of collagenase. LAP, isolated from culture medium of squamous cell carcinoma of prostate in rats, had been purified to homogeneity by adsorption chromatography through the controlled pore glass beads (CPG-250), gel filtration through Sephadex G-150 and ion-exchange chromatography through DEAE (diethylaminoethyl)-cellulose. The M Wt of LAP was estimated about 140,000 by gel filtration and 70,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Its isoelectric point was 4.5. The pH optimum for L-leucyl-2-naphthylamide (LNA) was in the range of 7.5 to 8.0. The Km value for LNA was 0.11 mM. The LAP activity was inhibited by cysteine, EDTA, o-phenanthroline, bestatin and pepstatin. The inhibition by bestatin was competitive and the Ki value was caculated to be 6.0 uM. The anti-LAP IgG, produced in rabbits, was able to inhibit the LAP activity. By immunofluorescent staining method, LAP similar to collagenase was found in the tumor connective tissue stroma. This suggested that LAP was produced by the connective tissue stroma cells in response to the action of tumor cell growth. (no Refs)

77. THE MEMBRANE ASSOCIATED FORM OF PLASMINOGEN ACTIVATOR AND ITS CONVERSION TO A SOLUBLE, EXTRACELLULAR FORM: A NEW CONCEPT FOR ENZYME SECRETION IN MALIGNANT CELLS.

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Diss Abstr Int [B]; 42(11):4279-B 1982

Plasminogen Activator (PA) exists in two states, a membrane-bound, cell-associated form and a released, soluble form in several mammalian systems and in chick embryo fibroblasts (CEF) transformed by Rous sarcoma virus (RSV). In cultures of RSVCEF the cell-associated form of PA exists in a firmly-bound membrane state. This association is not an artifact of either culture conditions or the membrane isolation procedures but rather appears to represent the functional in situ position of cell-associated PA. In contrast the released form of PA exists in a soluble state not associated with membranes. The release of PA from cultures of RSVCEF was found to be inhibited by the protease inhibitor chymostatin. Conversion of the membrane-bound form of PA to the extracellular, soluble form appears to involve a proteolytic step since chemical reduction of the anti-proteolytic aldehyde moiety on chymostatin essentially eliminates its effect on PA release. A specific chymostatin-sensitive protease has been detected in membranes isolated from RSVCEF cultures. This proteolytic activity appears to be a new and as yet undescribed serine endopeptidase exhibiting phenylalanine specificity and a unique inhibitor profile. This RSVCEF endopeptidase is present in normal CEF and similar to PA increases upon transformation and tumor promoter treatment. The presence of this chymostatin-sensitive endopeptidase in RSVCEF coupled with the sensitivity of PA release to chymostatin suggests a role for this endopeptidase in the conversion of the membrane form of PA to the released form. (no Refs)

78. CHARACTERIZATION OF COMPOUNDS SHED FROM THE SURFACE OF HUMAN LEUKEMIC MYELOBLASTS IN VITRO.

Baker MA, Roncari DA, Taub RN, Mohanakumar T, Falk JA, Grant S
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Blood; 60(2):412-419 1982

Human leukemic myeloblasts shed glycoproteins from the cell surface during short-term in vitro culture. Shed surface glycoproteins yield a characteristic profile when studied by gel chromatography, isoelectric focusing, immune precipitation, and polyacrylamide gel electrophoresis. Isolation of immunologically active material yields a compound of approx 75,000-80,000 daltons, with an isoelectric point of pI 7.6 to 7.9. Various morphological subtypes of acute myelogenous leukemia shed these compounds, but they are most easily obtained from the more differentiated M2 and M4 types as compared to the undifferentiated M1 type. The shed compounds appear to be quantitatively and qualitatively different from compounds shed by other leukemic cells or nonleukemic cells. (Author abstract) (30 Refs)

79. FACTORS INFLUENCING THE SHEDDING OF SURFACE COMPONENTS OF EHRLICH CELLS (MEETING ABSTRACT).

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Fed Proc; 41(3):755 1982

Ehrlich ascites tumor cells, when incubated in vitro, spontaneously shed cell surface or glycocalyx components into the medium. Exposure of cells to trypsin at low concentrations (1-5 ug/ml; 5-30 min at 37C) increases both the rate and amount of glycocalyx material released. The SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoretograms of the glycocalyx isolated from spontaneously shedding cells and trypsinized cells are essentially identical. Exposure of cells to 3H2DIDS (4,4'-dithiocyano-2,2'-dihydrostilbene disulfonic acid) at 0-4C leads to preferential labeling of the glycocalyx. 3H2DIDS-labeled cells incubated at 37C for 1 hr spontaneously released 3.5% of the total cell protein and 47 of the total 3H2DIDS. Repeated washings and resuspensions of 3H2DIDS-labeled cells with cold phosphate buffered saline also released protein and 3H2DIDS-labeled material. The amount released with each wash was essentially constant consisting of 0.45% of the total protein. The amount of H2DIDS-labeled material (cpm/ml glycocalyx) decreased with each wash. When separated by SDS-PAGE these components were identical to those isolated from cells incubated at 37C. The glycocalyx removed as a result of repeated washings contained no cytochrome oxidase, essentially no diaphorase and low levels of lactic dehydrogenase. It is concluded that the material shed by Ehrlich cells is of surface origin and very labile. (no Refs)

80. IMMUNOHISTOCHEMICAL LOCALIZATION OF CHONDROITIN-4-SULFATE PROTEOGLYCAN (CS-PG) FROM SWARM RAT CHONDROSARCOMA (SRC) IN NORMAL AND DIABETIC KIDNEYS (MEETING ABSTRACT).

Klein DJ, Michael AF, Oegema TR, Brown DM
University of Minnesota, Minneapolis, MN, 55455
Fed Proc; 41(3):856 1982

The immunohistochemical reactivity of antisera to guanidine-HCl extracted CG-PG monomer from SRC was studied in normal and diabetic kidneys. Antisera generated in New Zealand white rabbits was reactive to 1:120 dilutions by ELISA (enzyme-linked immunospecific antibody). Cross reactivity with human plasma fibronectin was eliminated using a fibronectin affinity column. Normal rat cartilage and the original SRC showed bright fluorescence in the extracellular matrix. There was a unique localization of stain to the pericellular areas of developing chondrocytes. Normal rat kidney showed positive fluorescence along proximal tubule brush-borders without interstitial staining. Minimal mesangial reactivity was present and no glomerular or tubular basement membrane staining was found from streptozotocin diabetic rats. Glomeruli had increased mesangial staining. Thus, CS-PG immunohistochemically localizes to normal cartilage and the pericelluar area of developing chondrocytes, confirming biochemical evidence that CS-PG from SRC is similar to that present in embryonic and immature cartilage. In addition, chondroitin-4-sulfate localizes to glomerular mesangium with a differing intensity in the diabetic rat, suggesting abnormal turnover or synthesis of CS-PG in diabetes. (no Refs)

81. BIOSYNTHESIS IN VITRO OF II RELATED GSL FROM NEOLACTOTETRAOSYLCERAMIDE BY AN N-ACETYLGLUCOSAMINYLTRANSFERASE FROM MOUSE T-LYMPHOMA (MEETING ABSTRACT).

Basu M, Kyle JW, Basu S
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Fed Proc; 41(3):888 1982

We have previously shown the biosynthesis in vitro of nLcOse4Cer (Gal beta 1-4GlcNAc beta 1-3Gal beta-4Glc-Cer) in B- and T-lymphomas. The present studies are concerned with the characterization of a specific N-acetylglucosaminyltransferase isolated from a purified membrane preparation from the mouse T-lymphoma P-1798. The enzyme activity, which requires a 1:6 detergent to protein ratio and MnCl2 (5.0 mM) for optimal activity at pH 7.0, catalyzes the reaction: UDP-GlcNAc + nLcOse4Cer yields GlcNAc beta-nLcOse4Cer. The radioactive product, isolated and purified by column chromatography and TLC (thin layer chromatography), migrated as a single band between nLcOse5Cer and Forssman GSL (glycosphingolipid). The terminal [3H]GlcNAc of the product was cleaved only 30-40% by jack bean and papaya beta-hexosaminidases. Further studies revealed that the rates of incorporation of [3H]GlcNAc into LacCer(Gal beta 1-4Glc-Cer) and LM1 (NeuGc(alpha 2-3)nLcOse4Cer) were 4% and 30%, respectively, of that with nLcOse4Cer. Incomplete cleavage of the radioactive product and incorporation of [3H]GlcNAc into LM1 suggest the presence of a beta (1-6) linked [3H]GlcNAc-containing radioactive product. A structural correlation between Ii GSL and the newly synthesized (in vitro) product is under investigation. (no Refs)

82. CELL SURFACE MEMBRANE CHANGES DURING THE DIFFERENTIATION OF HUMAN PROGRANULOCYTIC LEUKEMIA HL-60 CELLS (MEETING ABSTRACT).

Gupta SK, Felsted RL, Glover CJ, Bachur NR, Fischkoff S, Gallagher RE
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Fed Proc; 41(3):900 1982

The human promyelocytic leukemic cell line, HL-60, was induced to differentiate in vitro by treatment with dimethylsulfoxide and retinoic acid. Cell surface membrane proteins and glycoproteins were labeled with I-125 by the methods of lactoperoxidase/hydrogen peroxide or Iodo-gen and analyzed by two-dimensional polyacrylamide gel electrophoresis and autoradiography. A minimum of twelve cell surface proteins were unchanged, three proteins (MW 95,000; 87,000; and 77,000) were lost and seven new components (MW 280,000; 250,000; 162,000; 143,000; 58,000; 56,000; and 50,000) appeared during cell differentiation. Both chemical inducers gave similar results. The major labeled cell surface protein (MW 95,000) lost during cell differentiation correlated with the loss of cell transferrin binding and was identified as the transferrin receptor by affinity absorption of extracts of I-125 surface membrane-labeled cells with transferrin-Sepharose beads. This affinity purified component had molecular weights of 200,000 and 95,000 daltons under non-reducing and reducing conditions, respectively, confirming its dimer structure. Two-dimensional electrophoresis of cell surface membrane labeled normal granulocytes confirmed the absence of the transferrin receptor and the presence of several of the major new cell surface proteins identified during HL-60 maturation. (no Refs)

83. LOSS OF BASEMENT MEMBRANE COMPONENTS BY INVASIVE TUMORS BUT NOT BY THEIR BENIGN COUNTERPARTS (MEETING ABSTRACT).

Barsky SH, Siegal GP, Jannotta F, Liotta LA
Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD
Lab Invest; 46(1):7A 1982

Highly purified antibodies to three ubiquitous components of the basement membrane zone (type IV collagen, type V collagen, and laminin) were applied to both fresh frozen and fixed, paraffin-embedded permanent sections in a variety of invasive lesions, carcinomas in situ, and their 'look-alike' benign counterparts. These included such lesions as invasive breast carcinoma (scirrhous) in situ ductal carcinoma, and sclerosing adenosis; pancreatic carcinoma and pancreatitis; squamous cell carcinoma, Bowen's disease, and pseudoepitheliomatous hyperplasia; endometrial carcinoma and adenomyosis; prostatic carcinoma and benign prostatic hyperplasia; colonic adenocarcinoma and villous adenoma without invasion; and other lesions of the invasive, in situ, and noninvasive benign category. By both immunofluorescence and immunoperoxidase techniques, all benign and in situ lesions showed intact basement membrane with linear staining of type IV collagen, type V collagen, and laminin. In contrast, these three basement membrane components were absent in invasive carcinomas. In cases of in situ carcinoma with microinvasion, there was thinning, fragmentation, and disruption of the basement membrane in the foci of microinvasion. In some invasive tumors, especially well-differentiated carcinomas and in some sarcomas like synovial sarcoma, tiny discrete foci of extra-cellular basement membrane staining could be demonstrated with one or more of these antibodies, suggesting abortive attempts at reformation of basement membrane after initial invasion. (no Refs)

84. PLASMA MEMBRANE GLYCOPROTEINS OF TUMOUR CELLS IN ENZOOTIC BOVINE LEUKOSIS COMPARED WITH NORMAL BOVINE LYMPHOID CELLS.

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Acta Biol Med Ger; 41(2/3):145-160 1982

Plasma membranes from tumor cells in enzootic bovine leukosis and from normal bovine lymphoid cells of different sources have been isolated and characterized. The glycoprotein composition of the different membranes has been studied by SDS-PAGE and by analysis of the carbohydrate composition as well as by lectin binding of glycoprotein fractions obtained by phenol treatment of the membranes. No differences in the electrophoretical glycoprotein pattern could be detected comparing (nonmalignant) cells from persistent lymphocytosis and tumor cells, suggesting that malignancy is not associated with the gain or loss of a major glycoprotein. A 151.5 kD glycoprotein, present in membranes from normal lymph node lymphocytes, seems to be absent in the membranes of peripheral blood lymphocytes as well as of tumor cells. The loss of this glycoprotein might thus be associated with a loss of sessility of bovine lymphoid cells. The carbohydrate analysis and lectin binding of extracted glycoproteins revealed a decreased fucosylation accompanied by an increased exposure of galactose residues as well as a loss or a decreased complexity of N-glycosidically bound oligosaccharides in the tumor cell glycoproteins compared with those of normal cells. These findings are discussed with regard to disturbed growth regulation of leukosis tumor cells. (Author abstract) (49 Refs)

85. [14C]LEUCINE CHLOROMETHYLKETONE INTERACTION WITH SARCOMA 37 CELL PLASMA MEMBRANE COMPONENTS.

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Cancer Lett; 16(2):207-218 1982

Site(s) of leucine chloromethylketone (LCMK) interaction with S37 cells were identified to provide insight into the initial selective interaction between LCMK and the cell. The separation of the S37 ascites tumor cellular homogenate at 70,000 g for 16 hr on the ultracentrifuge yielded five visible fractions (Fractions A, B, C, D, and E) and a pellet. Fraction D contained 375 ug/ml of protein. Electrophoretic separation of 75 ug of protein from Fraction D exhibited a characteristic protein banding profile. Incubation of S37 cells for 5 min with the DL-[2-14C]LCMK at 22 C was followed by removal of the cells from the radiolabel and exposure to non-radiolabeled LCMK for 0, 15, 55, and 145 min. Subsequent Triton X-100 gel electrophoresis revealed a change of the radioactive label from an initial association with Band I to more negative proteins. Sodium dodecylsulfate electrophoresis of 37 ug of protein from Fraction D labeled for 5 min at 22 C with DL-[2-14C]LCMK, localized the radioactivity near the origin of the gel. By comparing the migration pattern of the radiolabeled proteins on a sodium dodecylsulfate polyacrylamide gel to a set of standard proteins, the mol wt of the labeled proteins was estimated to be between 100,000-200,000. Since a 5-min incubation of the S37 cells at 0 C with DL-[2-14C]LCMK and subsequent Triton X-100 gel electrophoresis of the plasma membrane fraction revealed no detectable radioactivity present in the gel, the binding of the radiolabeled material was temperature dependent. (33 Refs)

86. DIFFERENTIAL EFFECTS OF 2,4-DINITROPHENOL AND VALINOMYCIN (+K+) ON UNCOUPLER-STIMULATED ATPASE OF HUMAN TUMOR MITOCHONDRIA.

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Biochim Biophys Acta; 681(1):62-71 1982

The uncoupler-stimulated mitochondrial ATPase of four human tumors, mouse kidney, brain and fetal liver exhibited a characteristic behavior when preincubated with the H+-conducting uncouplers, dinitrophenol, CCCP, S-13 and gramicidin. The ATPase activity was considerably lower with preincubation than without. Preincubation with valinomycin (+K+), on the other hand, did not result in a significant decrease of the ATPase activity. These results may be contrasted with those obtained with liver or heart mitochondria, the ATPase activity of which did not suffer any loss when preincubated with dinitrophenol. The effect of preincubation with dinitrophenol on the tumor mitochondria could not be accounted for by dinitrophenol-induced Mg++ efflux, since the differential effects of dinitrophenol and valinomycin (+K+) remained even when ATPase activity was determined in presence of Mg++. Small amounts of ATP and ADP in the preincubation mixture containing dinitrophenol protected against the decay of the ATPase activity, implicating the exchangeable adenine nucleotides in the tumor mitochondria. In a model system where liver mitochondria were depleted of their adenine nucleotides, a lower ATPase activity was indeed obtained. However, direct determination of the concentrations of adenine nucleotides in dinitrophenol- and valinomycin-treated tumor mitochondria revealed only slight differences. (Author abstract) (23 Refs)

STUDIES OF PROTEIN TRANSLATION AND SYNTHESIS

87. VARIATIONS IN SOME MOLECULAR EVENTS DURING THE EARLY PHASES OF THE REUBER H35 HEPATOMA CELL CYCLE. III. ROLE OF PROTEIN SYNTHESIS IN THE INITIATION OF DNA SYNTHESIS AND THE MECHANISM OF STIMULATION OF PROTEIN SYNTHESIS BY SERUM.

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Biochimie; 64(6):411-418 1982

Induction of DNA synthesis by serum and amino acids has been investigated in cultured Reuber H35 hepatoma cells. Commitment to DNA synthesis was found to occur 6-8 hr before the actual start of this synthesis. The rate of initiation of DNA synthesis is proportional to the stimulation of protein synthesis by serum and/or amino acids. The increased protein synthesis is important for the proliferation only during the early period after serum addition. The withdrawal of serum and the inhibition by cycloheximide confirm this finding. Actinomycin D hardly influenced the early effect of serum on protein synthesis and it is concluded that the serum-stimulated protein synthesis is carried out on pre-existing mRNA's. The mechanism of stimulation of protein synthesis by serum has been studied by determination of the polyribosome size, the number of growing polypeptide chains, and the ribosomal transit time. The rate of the initiation of translation has been found to be specifically enhanced while the rate of elongation remained unchanged. Two-dimensional gel electrophoresis showed that the early stimulation of protein synthesis by serum involves all types of major cellular proteins, and no new proteins could be detected. (Author abstract) (38 Refs)

88. THE DISTRIBUTION PATTERN OF INFUSED L-[1-14C] LEUCINE INCORPORATED INTO PLASMA PROTEINS IN PATIENTS WITH VARIOUS STAGES OF COLORECTAL NEOPLASIA (MEETING ABSTRACT).

Claque MB, Carmichael MJ, Shenton B, Keir MJ, Johnston ID
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Gastroenterology; 82(5,part2):1034 1982

The percentage of L-[1-14C] leucine incorporated into plasma proteins during studies of body protein metabolism in surgical patients is increased in relation to disease activity and surgical stress. Although the synthetic rates of several plasma proteins are altered under such circumstances, the question arose as to whether the label was assimilated uniformly throughout, or limited to, certain plasma proteins (ie, acute phase reactants). To assess the distribution of label throughout the various plasma proteins with different levels of incorporation (6.7, 8.4, 11.1 and 13.4%) and disease activity (Dukes' classification A,C,D and D), up to 4 ml of plasma taken at the termination of infusions from 4 patients with colorectal neoplasia were eluted in fractions on an ACA 22 gel filtration column. Each fraction was assayed for total protein (Unicord recorder at optical density of 280 nm) and type (fused rocket electrophoresis into appropriate antibodies), and 1 ml aliquots were subjected to liquid scintillation counting. Results indicated that the pattern of distribution of counts did not change with disease activity, 55-60% of the label appearing in the albumin peak in each case. This would suggest that the mechanism of distribution involved is a general one, mediated either through changes in synthetic rates or specific radioactivity at the site of incorporation, and may be hormonally related. (no Refs)

89. DIBUTYRYL CYCLIC AMP TREATMENT MIMICS OVARIECTOMY: NEW GENOMIC REGULATION IN MAMMARY TUMOR REGRESSION.

Huang FL, Cho-Chung YS
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Biochem Biophys Res Commun; 107(1):411-415 1982

The possibility that the regression process of hormone-dependent tumors is regulated by a transcriptional event and that the regressions produced by hormone removal and dibutyryl cyclic (dc)AMP treatment are evoked by similar or identical mechanisms was investigated. The study presents the first evidence of differential gene expression during growth and regression of hormone-dependent dimethylbenz(a)anthracene-induced rat mammary carcinoma. Changes in translation products demonstrable during regression appeared to be specifically related to the hormone-independence of the tumors. As early as 6 hr post ovariectomy or dcAMP treatment (10 mg/200 g rat/day, sc), when there was no appreciable change in tumor size, the in vitro synthesis of several polypeptides instructed by poly(A)RNAs of tumors was either depressed or stimulated, indicating that these changes in gene expression are an early event in tumor regression. Both dcAMP and ovariectomy induced the same changes in the translation products. Thus estrogen and cyclic AMP may counteract each other at the nuclear level in the growth control of hormone-dependent mammary tumors. (17 Refs)

90. INCORPORATION OF NUCLEIC ACID AND PROTEIN PRECURSORS IN ENRICHED POPULATIONS OF PROLIFERATING NORMAL AND LEUKEMIC CELLS.

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Leuk Res; 6(3):365-370 1982

Nucleic acid and protein synthesis was studied in preparations enriched for normal immature myeloid cells and in leukemic samples with different proliferative activities. Bone marrow samples from seven normal subjects and from nine patients with newly diagnosed acute non-lymphocytic leukemia (ANLL) were used. According to the French-American-British system, two patients had M1 disease, two M2, three M4, one M5, and one M6. ANLL samples obtained from the bottom fraction of the sedimentation chamber contained 88 +-10% blastic cells. The normal and leukemic samples were comparable with respect to the percentage of S-phase cells and thymidine incorporation. ANLL samples contained 30.4 +-6.6% cells in S-phase and normal immature myeloid cells, 29.9 +-6.1%. Thymidine incorporation was 22,200 +-3,900 counts/min in ANLL and 17,500 +-2,900 counts/min in normal immature myeloid cells. The uridine incorporation of leukemic blasts was 49,000 +-8,500 counts/min and of normal immature myeloid cells, 24,000 +-4,900 counts/min. The leucine incorporation of ANLL samples was 6,100 +-800 counts/min and of immature myeloid cells 2,100 +-400 counts/min. The differences in uridine and leucine incorporation of normal and leukemic cells were statistically significant. Samples collected from the top of the sedimentation chamber contained 3.6 +-1.7% S-phase and 0.5 +-0.2% G2 + M-phase cells. Thymidine, uridine, and leucine incorporation was 1,100 +-400, 15,700+-3,200, and 2,600+-400 counts/min, respectively. Results showed that non-lymphocytic leukemic blast cells have a two-fold increase in RNA synthesis and a three-fold increase in protein synthesis compared to enriched samples of normal early myeloid cells with the same proliferative activity. (17 Refs)

91. O-GLYCOSYLATION OF THE MAJOR GLYCOPROTEIN OF THE MAT-B1 SUBLINE OF THE 13762 RAT ASCITES MAMMARY ADENOCARCINOMA (MEETING ABSTRACT).

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Fed Proc; 41(3):665 1982

To determine the subcellular site of O-glycosylation of proteins, we have examined the synthesis of ASGP-1, the predominant glycoprotein of 13762 mammary ascites tumor cells. The precursor of ASGP-1 (pre ASGP-1) can be isolated from galactose- or leucine-labeled cells by its association with peanut agglutinin (PNA)-bound agarose and elution by galactose. Pre-ASGP-1 has a lower density and a smaller molecular weight than ASGP-1. The processing of pre-ASGP-1 to ASGP-1 was demonstrated by pulse-chase analysis and gel filtration. The kinetics of incorporation of leucine into a PNA-binding component showed no lag, suggesting that glycosylation occurs concomitantly with protein synthesis. Polysomes obtained from uracil-labelled cells bound to PNA-agarose, permitting isolation of a t-RNA-associated glycoprotein. It has approximately the same elution weight as galactose-labeled pre-ASGP-1 on Sepharose CL-2B. These results suggest that the rough endoplasmic reticulum is the site of galactose attachment. (no Refs)

92. INDUCTION OF GP-2 MESSENGER RNA IN F9 CELLS BY RETINOIC ACID AND DIBUTYRYL CYCLIC AMP (MEETING ABSTRACT).

Durkin ME, Chung AE
University of Pittsburgh, Pittsburgh, PA, 15260
Fed Proc; 41(3):683 1982

The nullipotent embryonal carcinoma cell line F9 can be induced to undergo differentiation when treated with retinoic acid and dibutyryl cyclic AMP. After 48 hours of growth in medium containing 2 x 10-7M retinoic acid and 1mM dibutyryl cyclic AMP, the synthesis of the basement membrane-related glycoproteins GP-1, GP-2 (laminin), and entactin is switched on in F9 cells. The increase of GP-2 synthesis has been demonstrated by immunoprecipitation of GP-2 from cell lysates and growth medium of induced cells. Messenger RNA was extracted from induced and uninduced cells and used to program a rabbit reticulocyte lysate in vitro translation system. When translation products directed by induced F9 RNA were immunoprecipitated with GP-2 antiserum and the immunoprecipitates analyzed by SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis, a band was observed which has a mobility similar to that of unglycosylated precursors of GP-2 seen in endodermal cells treated with tunicamycin. This band was not visible in the immunoprecipitates of translation products directed by uninduced F9 RNA. These results suggest that the increase in GP-2 synthesis caused by retinoic acid and dibutyryl cyclic AMP is due to an increase in translatable GP-2 mRNA (messenger RNA). (no Refs)

93. POLYSOMAL DISTRIBUTION OF MRNA IN THE CELL CYCLE OF MOUSE S-180 ASCITES CELLS (MEETING ABSTRACT).

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University of Louisville, Louisville, KY, 40292
Fed Proc; 41(3):1285 1982

Mouse sarcoma-180 ascites cells contain a limited population of cytoplasmic messenger ribonucleoprotein (mRNP) whose translation appears to be restricted in vivo as well as in vitro. To determine if this population of mRNP changes during the cell cycle stages by centrifugation through ficoll gradients. The distribution of cells able to incorporate (3H)-thymidine into DNA suggests that fractionation of ascites cells on the basis of size will yield cell cycle enriched populations. A bi-modal distribution of cells with maximal protein synthesis activity also supports this view. Newly synthesized polypepides examined by gel electrophoresis show some quantitative changes during the ascites cell cycle. The polysomal distribution of total and newly synthesized poly(A)-containing RNA was examined in cell cycle enriched fractions. The results suggest a small shift in mRNA from the non-polysomal to the polysomal region of a sucrose gradient in cells that are smaller than those in S phase (presumably G1 phase cells). There is also a substantial change in the population of non-polysomal mRNA detected by translation in vitro. In this case however, cells smaller than those in S phase (presumably G1 phase cells) contain the limited population of abundant mRNAs, while these mRNAs are absent or present in reduced amounts in the presumed G2 phase cells. (no Refs)

94. EFFECT OF INSULIN AT DILUTION ENDPOINT CONCENTRATIONS ON MACROMOLECULAR SYNTHESIS IN NORMAL MOUSE MAMMARY AND HUMAN BREAST CANCER EPITHELIAL CELLS.

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Biochim Biophys Acta; 720(4):346-355 1982

Employing defined media conditions, the insulin sensitivities of mouse mammary gland epithelial cells in primary culture and MCF-7 human mammary epithelial cells were determined. Insulin stimulated the rates of [3H]uridine incorporation into RNA and [3H]leucine incorporation into protein in both primary mouse mammary gland epithelial cell cultures and MCF-7 cell cultures at concentrations approximating the dilution endpoint of the hormone (10(-21) M). Insulin stimulated the rate of [3H]thymidine incorporation into DNA in primary mouse mammary gland epithelial cells at the dilution endpoint concentrations. However, MCF-7 cells required insulin concentrations 100-1,000x that necessary in mouse mammary epithelial cultures to elicit an increased rate of [3H]thymidine incorporation into DNA. Evidence is presented which suggests that the increased rates of uptake of [3H]uridine, [3H]thymidine and [3H]leucine into their respective precursor pools is not responsible for the apparent stimulation of RNA, DNA and protein synthesis. (Author abstract) (24 Refs)

95. CHARACTERIZATION OF GAMMA-MELANOTROPIN-LIKE IMMUNOREACTIVITY AND ITS SECRETION IN AN ADRENOCORTICOTROPIN-PRODUCING MOUSE PITUITARY TUMOR CELL LINE.

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Endocrinology; 111(2):418-424 1982

A putative hormone in the N-terminal region of the ACTH/beta-endorphin (beta-EP) precursor protein, gammaMSH, was studied by RIA with an antiserum against gamma3MSH in ACTH-producing mouse pituitary tumor cells, AtT-20/D16v. Serial dilution of the culture medium or the cell extract gave parallel lines to the standard curve on the RIA for gammaMSH. Rat median eminence extracts enhanced the release of gammaMSH-like immunoreactivity (gammaMSH-LI) concomitant with ACTH-like immunoreactivity (ACTH-LI) and beta-EP-like immunoreactivity (beta-EP-LI). Dexamethasone suppressed the release of gamma-MSH-LI as well as ACTH-LI and beta-EP-LI. Gel exclusion chromatography of the culture medium and the cell extract has revealed that gamma-MSH-LI consists of two peaks; one eluted near the elution position of beta-lipotropin and the other near the elution position of beta-EP. There was no peak corresponding to the elution position of synthetic gamma3MSH. However, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has demonstrated that gammaMSH-LI migrated at five positions with mol wt of 31K, 21-23K, 16-17K, 13-14K, and 3.8K, respectively. The 31K gammaMSH coincided with the migration position of 31K ACTH or 31K beta-EP, and 21-23K gammaMSH coincided with the position of 21-23K ACTH on SDS-PAGE. The 16-17K gammaMSH coincided with the mouse 16K fragment (reported by Eipper and Mains) of ACTH-beta-lipotropin precursor protein in the migration in SDS-PAGE and in immunoreactivity to anti-gammaMSH antiserum. [3H]Glucosamine was incorporated into 16K, 13K, and 3.8K gammaMSH. These results suggest that AtT-20/D16v cells produce gammaMSH-LIs with mol wt of 31K, 21-23K, 16-17K, 13-14K, and 3.8K, and they are secreted concomitantly with ACTH-LI and beta-EP-LI. (Author abstract) (28 Refs)

96. SERUM PROTEIN SYNTHESIS BY SOMATIC CELL HYBRIDS.

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Dept. Human Genetics, Univ. Pennsylvania Sch. Medicine, Philadelphia, PA, 19104
Somatic Cell Genet; 8(4):433-449 1982

Serum protein synthesis by rodent hepatoma x fibroblast, hepatoma x teratocarcinoma, and hepatoma x hepatoma somatic cell hybrids was analyzed by Lurell (rocket) and crossed immunoelectrophoresis. Of 22 hepatoma x fibroblast hybrids, 19 synthesized transferrin. Albumin was only synthesized at detectable levels by one hybrid. Alpha-fetoprotein synthesis could not be detected for any of the eight hybrid clones between the mouse hepatoma cell line Hepa 1a and the rat and mouse fibroblasts. Fibrinogen synthesis, or that of five other serum proteins synthesized by the rat hepatoma cell line Faza could not be detected for nine hybrid clones between Faza and rat or mouse fibroblasts. Reduced transferrin and albumin synthesis by rat hepatoma (Faza) x mouse hepatoma (Hepa 1) hybrids in comparison with parental cells indicated that causes other than differentiation state of the cells played a role in limiting plasma protein synthesis in somatic cell hybrids. Specific repression of the transferrin gene may be involved. Alpha-fetoprotein synthesis occurred in the Faza x Hepa 1 hybrids, yet neither parental strain produces this protein. The absence of transferrin and albumin synthesis by hepatoma x teratocarcinoma hybrids may be due to repression by the teratocarcinoma gene. Transferrin is apparently unique among serum proteins in being synthesized by hepatoma x fibroblast cell hybrids. (23 Refs)

97. SYNTHESIS OF THE VIRAL GLYCOPROTEIN OF ROUS-ASSOCIATED VIRUS-2.

Enrietto PJ, Purchio AF, Erikson RL
Imperial Cancer Res. Fund, London WC2A 3PX, England
J Virol; 43(2):756-760 1982

The synthesis of the viral glycoprotein of Rous-associated virus-2 was studied in vitro in a cell-free system programmed with viral RNA and supplemented with dog pancreas membranes. The protein synthesized was related structurally and immunologically to those found in Rous-associated virus-2-infected chicken embryo fibroblasts. This work confirms and extends earlier work on the nature and synthesis of viral glycoproteins. (Author abstract) (24 Refs)

98. IDENTIFICATION OF PROTEINS ENCODED BY THE GAZDAR MURINE SARCOMA VIRUS GENOME BY IN VITRO TRANSLATION AND COMPARISON WITH MOLONEY MURINE SARCOMA VIRUS 124.

Papkoff J, Hunter T
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Virol; 43(2):533-543 1982

The gene products of Gazdar murine sarcoma virus (Gz-MuSV) were identified by in vitro translation of Gz-MuSV virion RNA. An overlapping set of proteins with approximate mol wt of 37,000 (37K), 33K, 24K, and 18K were synthesized from the transforming gene of Gz-MuSV, v-mosGz. In addition, Gz-MuSV-specific RNA directed the in vitro synthesis of a 62K gag gene protein and a 37.5K env gene-related product. The Gz-MuSV-specific in vitro translation products were compared with the in vitro translation products of M-MuSV 124, an independent isolate with a similar v-mos gene. This analysis showed that the 62K Gz-MuSV gag gene protein and the 37K, 33K, 24K, and 18K v-mosGz proteins were almost identical to the M-MuSV 124 62K (gag) and 37K, 33K, 24K, and 18K (v-mosMo) proteins that we previously identified and characterized. The 37.5K env gene product from Gz-MuSV does not have a correlate in the M-MuSV 124 translation products. These results were analyzed in the context of expectations based on similarities and differences in genetic organization of these two viral genomes. (Author abstract) (36 Refs)

99. SPECIFIC SECRETION OF POLYPEPTIDES FROM CELLS INFECTED WITH MYXOMA VIRUS.

Pennington TH, Carnegie GE, Alcock SR
Dept. Bacteriology, Univ. Aberdeen, Univ. Medical Buildings, Foresterhill, Aberdeen AB9 2ZD, Scotland
J Gen Virol; 61(part1):115-120 1982

The polypeptides secreted from cells infected with myxoma virus have been studied. Three virus-induced polypeptides were detected. One major and one minor polypeptide were synthesized and secreted in the absence of virus DNA synthesis: one minor polypeptide was not detected in the medium under such conditions. All these polypeptides were glycosylated and one was sulfated. Two precipitin lines were seen in Ouchterlony tests examining medium from infected rabbit cells and using serum from rabbits convalescent from myxoma virus infections. These antigens were unrelated to the virus-specific antigens released from cells infected with vaccinia virus. No major differences were detected in comparisons between the polypeptides secreted from cells infected with virulent and relatively avirulent strains of myxoma virus. (Author abstract) (12 Refs)

100. ANTITUMOR AGENTS LIV: THE EFFECTS OF DAPHNORETIN ON IN VITRO PROTEIN SYNTHESIS OF EHRLICH ASCITES CARCINOMA CELLS AND OTHER TISSUES.

Liou YF, Hall IH, Lee KH
Div. Medicinal Chemistry, Sch. Pharmacy, Univ. North Carolina at Chapel Hill, Chapel Hill, NC, 27514
J Pharm Sci; 71(7):745-749 1982

Daphnoretin has been shown to suppress Ehrlich ascites carcinoma growth in mice. One of the modes of action of the drug is to block protein synthesis; however, the inhibition of protein synthesis requires a higher concentration of drug than the inhibition of DNA synthesis. The inhibition of protein synthesis appears to occur during the elongation step with the drug preferentially binding to free ribosomes not engaged in active protein synthesis. (Author abstract) (23 Refs)

EFFECTS OF EXOGENOUS AGENTS ON PROTEIN METABOLISM

101. ENHANCEMENT OF HUMAN CHORIONIC GONADOTROPHIN PRODUCTION BY ANTIMETABOLITES.

Browne P, Bagshawe KD
Dept. Medical Oncology, Charing Cross Hosp., Fulham Palace Road, London W6 8RF, England
Br J Cancer; 46(1):22-29 1982

The action of methotrexate, actinomycin D, bleomycin, vincristine and hydroxyurea on the production of human chorionic gonadotrophin (hCG) by a choriocarcinoma cell line (BeWo) has been studied. hCG production per unit of cell protein was increased, and this was a continuing process only halted by cell death. The proportion of hCG-producing cells in the population increased from approx 5% to a max of approx 40% during incubation with vincristine. A possible explanation for these and related observations is that cytotoxic agents promote the differentiation of cytotrophoblast to syncytium. A similar mechanism could contribute to the unusual sensitivity of choriocarcinoma to cytotoxic agents. (Author abstract) (23 Refs)

102. PHORBOL ESTERS MODULATE THE BINDING OF SEVERAL PEPTIDE HORMONES TO PITUITARY CELLS (MEETING ABSTRACT).

Osborne R, Tashjian AH
Department of Pharmacology, Harvard Medical School, Boston, MA
Toxicologist; 2(1):65 1982

Phorbol esters (PE) are potent tumor promoters which have been shown to alter specifically the binding of epidermal growth factor (EGF) to diverse cell types in culture. In GH4C1 cells, a clonal strain of rat pituitary cells, PE stimulate the synthesis and release of prolactin and inhibit growth hormone production after binding to specific PE receptors. GH4C1 cells also have specific membrane receptors for EGF, thyrotropin-releasing hormone (TRH), and somatostatin (SRIH). Treatment with PE decreased the binding of EGF, TRH and SRIH to GH4C1 cells. 12-O-Tetradecanoyl-13-phorbol acetate (TPA) at 100 ng/ml decreased 125I-EGF binding to 15% of control within 1 hr. By Scatchard analysis of 125I-EGF binding data, a 1-hr pretreatment with TPA decreased both the apparent affinity and the number of 125I-EGF receptors. After 4 hr of treatment, TPA decreased 3H-TRH binding to 35% of control due to a decrease in affinity with no change in the number of TRH receptors. After 24 hr, TPA also decreased 125I-Tyr1-SRIH binding to 40% of control. Effects of TPA on peptide binding were temperature dependent, occurring at 37 degrees but not 4C, and reversible. The potency order for decreased peptide binding was TPA greater than phorbol-12,13-didecanoate (PDD) greater than phorbol-12,13-dibenzoate, while phorbol and 4 alpha-PDD were inactive, which parallels the activity of these compounds as tumor promoters. Thus, unlike findings reported in certain other cells, the actions of PE on peptide ligand binding in GH4C1 cells were not specific for EGF. (no Refs)

103. A PHORBOL ESTER TUMOR PROMOTER INDUCES CHANGES IN THE EXPRESSION OF IMMUNOGLOBULINS AND DR ANTIGENS IN HUMAN LYMPHOBLASTOID CELLS.

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J Immunol; 129(2):539-544 1982

The present study indicates that 12-O-tetradecanoyl phorbol 13-acetate (TPA) can induce either increases or decreases in the relative expression of membrane-associated IgM and IgD as well as the expression of other markers of lymphocyte differentiation on human B lymphoblasts, depending on the clone studied. The changes in membrane Ig expression are apparent within 24 hr and are maximal at about 48 hr after the addition of the compound. In clone BL, TPA decreases the membrane IgM/IgD ratio as determined by the percentage of cells bearing these isotypes and by their densities on single cells. In clone MW-E, TPA has the opposite effect. Although these changes are followed by an increase in Ig secretion by MW-E cells, measured by immunofluorescence and the protein A plaque assay, There is no induction of Ig secretion in BL cells. In addition, TPA increases the expression in BL of an additional B lymphocyte marker, the D region-related (DR) antigens, while having the opposite effect in MW-E. In two sublines of Daudi, a membrane IgM-positive, IgD-negative, nonsecreting cell line, TPA treatment resulted in the expression of membrane IgD without the induction of Ig secretion. Thus, the TPA-induced changes in the IgM/IgD ratio and Dr antigen expression are inversely related and are not due to differential proliferation of subpopulations. Taken together, it appears that, depending on the particular clone of B lymphoblasts studied, TPA can induce or inhibit a coherent program of B lymphocyte maturation. (Author abstract) (52 Refs)

104. CHARACTERIZATION OF VARIANT NEUROBLASTOMA CLONES WITH MISSING OR ALTERED SODIUM CHANNELS.

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Mol Pharmacol; 22(1):196-203 1982

The effect of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatment upon the frequency and phenotype of toxin-resistant clones derived from clone N18 of mouse neuroblastoma C1300 were studied, and both parental and variant clones were examined for synthesis of the alpha-subunit of the sodium channel. MNNG in the concentration range 0.25 to 2 ug/ml increased up to 16-fold the frequency of sodium channel-deficient clones which lack functional Na channels, scorpion toxin-resistant clones with modifications of scorpion toxin receptor site, and resistant clones having sodium channels with parental properties. One- or two-dimensional gel electrophoresis showed no evidence of synthesis of the alpha-subunit of the sodium channel, consistent with the conclusion that the gene coding for this protein is not expressed or is poorly expressed in the sodium channel-deficient clones. Of three scorpion toxin-resistant clones analyzed here, one seemed to have a sodium channel with clearly altered properties. Two-dimensional gel electrophoresis demonstrated the presence of the alpha-subunit of the sodium channel. The frequency of appearance of variants with structural gene alterations in the sodium channel is much less than the overall rate of appearance of neurotoxin-resistant variants, but it may be comparable to the frequency of mutations in the gene for hypoxanthine-guanine phosphoribosyl transferase as assessed by 6-thioguanine resistance. (24 Refs)

105. EFFECTS OF RETINOIC ACID ON DIFFERENTIATION OF CHORIOCARCINOMA CELLS IN VITRO (MEETING ABSTRACT).

Chou JY
National Institutes of Health, Bethesda, MD, 20205
Fed Proc; 41(3):683 1982

Choriocarcinoma cells secreted no immunoreactive pregnancy-specific beta 1-glycoprotein (PS betaG) in vitro. These cells can be induced to synthesize low levels of PS betaG by retinoic acid, 8-Bromo-cAMP (8BrcAMP), cholera toxin, methyl isobutyl xanthine (MIX), and 5-Bromo-2'-deoxyuridine (BrdUrd). Simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave synergistic induction of PS betaG. Simultaneous addition of retinoic acid and BrdUrd failed to given even additive induction. In addition to stimulating PS betaG production, retinoic acid increased the production of human chorionic gonadotropin (hCG) and its alpha subunits (hCGalpha) by choriocarcinoma cells. Simultaneous addition of retinoic acid along with 8BrcAMP, cholera toxin, or MIX gave additive induction for hCG and hCGalpha. Passage of choriocarcinoma cells in medium containing retinoic acid induced a stable altered phenotype characterized by elevated levels of PS betaG, hCG, and hCGalpha. These retinoid-treated choriocarcinoma cells remained responsive to 8BrcAMP or compounds that increase intracellular cAMP concentrations and to BrdUrd: the production of PS betaG and hCG was greatly stimulated by 8BrcAMP, cholera toxin, or MIX and the production of hCG and hCGalpha was greatly inhibited by BrdUrd. However, the production of hCGalpha was only slightly induced by these cAMP modulators and the production of PS betaG was not increased by BrdUrd. Our data suggest that retinoic acid plus compounds that elevate cAMP concentration may induce differentiation of choriocarcinoma cells in vitro. (no Refs)

106. LUNG PROTEASE ACTIVITY STIMULATED BY BLEOMYCIN (MEETING ABSTRACT).

Roffman S, Cantor J, Osman M, Mandl I, Turino GM
Columbia University, New York, NY
Fed Proc; 41(3):688 1982

The responses of lung interstitium to various forms of injury may be mediated by cathepsin D and non-elastase serine proteases. Hamsters (female, 110 grams) received 1 unit of bleomycin intratracheally. After six days, lung tissue homogenates, lavaged cells and fluid exhibited elevated levels (greater than 50%) of [3H]tosylarginine methyl esterase (TAME) activity at neutral pH and elevated (greater than 80%) levels of [3H]acetylhemoglobin (AcHb) hydrolytic activity (pH 3.5). TAME hydrolysis was inhibited by phenylmethyl sulfonyl fluoride, leupeptin, trasylol, soy and lima bean trypsin inhibitors. AcHb activity was completely inhibited by pepstatin identifying it as cathepsin D-like. Only cathepsin D activity remained elevated at day 14 although treated and control lung homogenates exhibited both types of protease activities. Specific activity of cathepsin D was much greater in parenchyma than in lavaged cells, suggesting it is elaborated by parenchymal cells. Lung tissue injury by these proteases may constitute part of the mechanism leading to interstitial fibrosis induced by bleomycin injury. Cathepsin D elevation in bleomycin injured lung may also contribute to interstial edema through its release of the vasoactive peptide, leukokinin, believed to increase vascular permeability and fluid accumulation in inflammatory disease. (no Refs)

107. BLEOMYCIN-INDUCED INCREASE OF COLLAGEN TURNOVER IN IMR-90 FIBROBLASTS: AN IN VITRO MODEL OF CONNECTIVE TISSUE RESTRUCTURING DURING LUNG FIBROSIS.

Sterling KM, DiPetrillo TA, Kotch JP, Curtoneo KR
Dept. Biochemistry, Coll. Medicine, Univ. Vermont, Burlington, VT, 05405
Cancer Res; 42(9):3502-3506 1982

Late-log-phase IMR-90 human fetal lung fibroblasts were incubated with bleomycin sulfate for 48 hr. The culture medium was removed and replaced with serum-free medium and [5-3H]proline. The cells were than incubated for increasing time intervals. The cells and culture medium were collected, and radioactive proline incorporated into collagen and noncollagen protein was determined. Intracellular collagen syntheses was selectively increased. Furthermore, polysomes isolated from bleomycin-treated cells synthesized significantly more collagen in the wheat germ lysate than did control polysomes. Prolyl hydroxylase activity was also increased significantly in the bleomycin-treated cells. Free and peptide-bound radioactive hydroxyproline in the cells and medium was greatly increased in bleomycin-treated cells, which indicates increased collagen degradation. The results demonstrate that, although collagen synthesis in lung fibroblasts is increased by bleomycin, the newly synthesized collagen is rapidly degraded in both the cell layer and the medium. This increased collagen degradation may be responsible for the remodeling which takes place during lung fibrosis. (Author abstract) (38 Refs)

108. ADENOSINE DEAMINASE FROM SENSITIVE AND DEOXYCOFORMYCIN-RESISTANT RAT HEPATOMA CELLS: PURIFICATION AND CHARACTERIZATION (MEETING ABSTRACT).

Hunt SW, Labant MC, Hoffee PA
University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261
Fed Proc; 41(3):904 1982

Rat hepatoma cells resistant to deoxycoformycin (dCF) exhibit up to a 20-fold increase in adenosine deaminase (ADA) activity compared to the sensitive parental cell line. In order to characterize the enzymes synthesized by these cells, ADA was purified to apparent homogeneity from sensitive and dCFr cultures. ADA was also purified from rat liver and was used for the production of specific antibody. The enzymes from all three sources were found to be immunologically indistinguishable by quantitative immunoprecipitation and double diffusion analyses. No differences between the enzymes were noted with regard to the Km for adenosine or the Ki for dCF. All three enzymes had an apparent MW of 45,000 as determined by SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis. By all parameters examined, the ADA proteins synthesized by sensitive and dCFr cells are similar, if not identical. These data indicate that the increased ADA activity in dCFr cells is due to an increase in the amount of ADA protein molecules. Comparison of the amounts of newly synthesized protein in sensitive and dCFr cells supported this conclusion. In addition, dCFr cells were found to express elevated amounts of a second protein. (no Refs)

109. A CYTOCHROME P-450 ISOZYME WITH HIGH AFFINITY FOR DIMETHYLNITROSAMINE: INDUCTION BY PYRAZOLE AND OTHER NITROGENOUS COMPOUNDS (MEETING ABSTRACT).

Tu YY, Yang CS
Department of Biochemistry, New Jersey Medical School, Newark, NJ, 07103
Fed Proc; 41(3):1402 1982

Treatment of rats with pyrazole and other nitrogenous compounds elevated the hepatic microsomal dimethylnitrosamine demethylase activity (DMNd). The highest inducton was observed with pyrazole. DMNd was induced 3-4 fold but some classical monooxygenase activities were not induced. The increased DMNd was accompanied by the appearance of a new microsomal protein shown as a 52,000 dal band in polyacrylamide gel electrophoresis. This protein species was decreased by in vivo treatment with $CoCl_2$ which also decreased DMNd, suggesting the involvement of a hemoprotein in the induction. The DMNd of the pyrazole-induced microsomes showed a Km of 61 uM and Vmax of 12.1 nmol/min/mg which were clearly different from those of the control microsomes. Limited tryptic digestion cleaved the active site of NADPH-cytochrome P-450 reductase and also decreased the microsomal DMNd. The DMNd was regained by preincubating the microsomes with purified reductase. This indicates that the reductase is an essential component enzyme of the DMNd in both the control and pyrazole-induced microsomes. The results suggest that pyrazole induces a P-450 isozyme which has high affinity and turnover for dimethylnitrosamine and probably for other nitrosamines. (no Refs)

110. REGULATION OF GLOBIN GENE EXPRESSION IN FRIEND ERYTHROLEUKEMIC CELLS.

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Diss Abstr Int [B]; 42(11):4310-B 1982

The experiments consisted of: (1) those dealing with Friend erythroleukemic cells which were capable of undergoing spontaneous erythroid differentiation, as measured by the synthesis of hemoglobin, and (2) those dealing with Friend cells which differentiate in the presence of an added inducer. The results indicate that (1) at least three mechanisms exist whereby inducers act in turning on erythroic development in Friend cells, (2) the expression of beta major and beta minor globin genes are independently controlled, and (3) variations in the levels of beta major and beta minor globin chains expressed in response to specific chemical inducers is the result of some form of transcriptional rather than translational regulation. Using drug resistant, non-inducible variant clones, it was possible to demonstrate the existence of at least three distinct classes of inducers represented by: (A) polar planar compounds; (B) fatty acids and (C) hemin. Based on the electrophoretic profiles of Triton X-100, acid-urea, polyacrylamide slab gels of hemin treated cells, and DMSO induced cells incubated with or without allylisopropylacetamide, hemin was shown to be specifically involved in the regulation of beta minor globin chains. (no Refs)

111. INTERINDIVIDUAL VARIATION IN BENZO(A)PYRENE METABOLISM AND COMPOSITION OF ISOENZYMES OF CYTOCHROME P-450 AS REVEALED BY SDS-GEL ELECTROPHORESIS OF HUMAN LIVER MICROSOMAL FRACTIONS.

Ekstrom G, Von Bahr C, Glaumann H, Ingelman-Sundberg M
Dept. Chemistry, Karolinska Inst., Huddinge, Sweden
Acta Pharmacol Toxicol (Copenh); 50(4):251-260 1982

Human liver microsomal fractions from 13 different individuals were characterized with respect to SDS-polyacrylamide gel electrophoretic profiles and regiospecificity in the metabolism of the polyaromatic hydrocarbon benzo(a)pyrene. Pronounced interindividual differences in the composition of microsomal proteins in the mol wt region of Mr = 49,000-60,000 were found. Furthermore, gel electrophoresis combined with staining for peroxidase activity indicted most of the variations among the profiles of microsomal proteins being attributed to interindividual differences in the composition of isoenzymes of cytochrome P-450. One type of human liver microsomal fraction was selectively induced in specimens obtained from patients with known regular drug intake before death and correlated well in mol wt to the phenobarbital-inducible form of cytochrome P-450 in the rabbit. Large variations among the human liver microsomal samples were also seen in the benzo(a)pyrene metabolism. The results indicate the presence of 7-8 different forms of cytochrome P-450 in human liver microsomes and that the interindividual variations seen in drug metabolism may at least in part be explained by interindividual variations in the distribution of these isoenzymes. (Author abstract) (24 Refs)

112. DOSE DEPENDENT INDUCTION OF RAT LIVER MICROSOMAL CYTOCHROME P-450 AND MICROSOMAL ENZYMATIC ACTIVITIES AFTER INHALATION OF TOLUENE AND DICHLOROMETHANE.

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Acta Pharmacol Toxicol (Copenh); 51(2):108-114 1982

Sprague-Dawley rats were exposed, by inhalation, to toluene and dichloromethane (500, 1,500 or 3,000 ppm) and to benzene (1,500 ppm) for 3 days. Toluene and benzene increased the concentration of liver microsomal cytochrome P-450. A dose dependent increase in the in vitro liver microsomal formation of several metabolites of biphenyl and benzo(a)pyrene was observed for both dichloromethane and toluene. At the highest dose-level the increase in the in vitro formation of benzo(a)pyrene-7,8-dihydrodiol was more than three-fold for both dichloromethane and toluene whereas the formation of benzo(a)pyrene-4,5-dihydrodiol increased more than five-fold following exposure to toluene but less than two-fold after exposure to dichloromethane. Our results suggest that dichloromethane and toluene can modify the metabolism and thereby the toxicity of other environmental contaminants. (Author abstract) (30 Refs)

113. SPONTANEOUS PRODUCTION OF ALPHA- AND BETA-INTERFERON IN HUMAN LYMPHOBLASTOID AND LYMPHOMA CELL LINES.

Adolf GR, Haas OA, Fischer P, Swetly P

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Arch Virol; 72(3):169-178 1982

A large number of human hematopoietic cell lines was examined for spontaneous production of interferon. Unconcentrated culture supernatants from 70 out of 71 B-lymphoblastoid cell lines contained considerable amounts of interferon (median titer 22 units per ml); a few lines produced more than 100 units/ml with peak values up to 500 units/ml. In contrast, only one B-lymphoma line out of 18 genuine lymphoma, myeloma, and leukemia cell lines tested spontaneously produced small amounts of interferon. Following treatment with 5-bromodeoxyuridine (BrdUrd), interferon was produced without further induction in most B-lymphoid cell lines, but not in any of the non-B, non-T, T-lymphoid or myeloid lines examined. Modulation of spontaneous interferon production by chemicals (sodium butyrate, dexamethasone, dimethylsulfoxide, a phorbol ester, and BrdUrd) was studied in more detail in three B-lymphoblastoid and four B-lymphoma cell lines. The patterns of responses observed were different for the action of different chemicals on a given cell line as well as between lymphoblastoid and lymphoma lines in general; furthermore, several lines of evidence suggest that chemicals can differentially influence spontaneous and virus-induced interferon production in a given cell line. The composition of spontaneously produced interferon was analyzed using antisera specific for HuIFN-alpha and HuIFN-beta. Interferons produced by untreated as well as BrdUrd-treated lymphoblastoid cells contained more than 95% IFN-alpha, whereas BrdUrd-treated lymphoma cells produced IFN-alpha as well as minor amounts (cell lines Namalwa and NC-37) or even over 90% of IFN-beta (Daudi). (Author abstract) (17 Refs)

DIETARY AND NUTRITIONAL EFFECTS

114. INCREASED PROTEIN TURNOVER IN CHILDREN WITH CANCER (MEETING ABSTRACT).

Kien CL, Camitta BM

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Pediatr Res; 16(4,part2):168A 1982

We are studying whether accelerated rates of protein turnover in normal and/or cancer cells might result in elevated energy requirements in cancer patients and thus promote cachexia. Using a single dose, [15N]-glycine technique, we assessed 15N enrichment of urine ammonia and calculated rates of whole body protein synthesis (S) and breakdown (B) in children who are healthy (H) (6) (5-17 yr) or had newly diagnosed leukemia or lymphoma (L) (8) (4-15 yr). The cancer patients showed significant 51-112% elevations in rates of S and B; however, the patients were not in a hyperalimentary state and actually had a lower protein intake than controls (two sample t test). The rates of S and B were also significantly higher in the L group using the Wilcoxon test. Some cancer patients may require high energy intake to maintain both high rates of protein synthesis and normal body composition. (1 Ref)

115. NUTRITION AND THE SURGICAL PATIENT.

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Dept. Surgery, Univ. Auckland Sch. Medicine, Auckland, New Zealand

Nutrition and the Surgical Patient. Edinburgh, Churchill Livingstone, Clinical Surgery International, Vol. 2, 323 pp., 1981.

Surgical nutrition is discussed with respect to practical application in clinical practice. Nutritional disorders, protein energy malnutrition, and assessment of protein energy malnutrition in surgical patients are discussed. Indications for nutritional therapy prior to and after major surgery are described. Practical aspects of enteral nutrition and iv nutrition are reviewed. Experience with iv hyperalimentation using solutions in which glucose is the sole non-protein energy source is described and the practical and theoretical advantages of using some fat as part of the non-protein energy source are presented. Complications of iv nutrition and their prevention, technical aspects of iv nutrition, and trace metals in iv nutrition are discussed. Results of iv nutrition in cancer and gastroenterological patients are reported. Hepatic, intestinal, and acute renal failure, treatment of burn injuries, gastrointestinal fistulas, and inflammatory bowel disease are described. The role of nutrition in patients with gastrointestinal fistulas and cancer and psychiatric problems encountered during iv nutrition are discussed. A variety of clinical manifestations of nutritional disorders that may occur in the surgical patient are discussed along with the underlying disease processes that give rise to them and the influence that they may have on prognosis. Management of the patient with established malignant disease where nutritional depletion has developed and can be reversed by enteral or parenteral nutrition is discussed. (935 Refs)

OTHER RELATED STUDIES

116. COVALENT BINDING OF PHOTO-OXIDIZED BENZ[A]ANTHRACENE AND RELATED CARCINOGENIC HYDROCARBONS TO PROTEIN.

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Cancer Lett; 16(2):219-225 1982

Formation of unique photo-oxidized products from benz[a]anthracene (BA), 7,12-dimethylbenz[a]anthracene (DMBA), and 3-methylcholanthrene (MC) in the presence of bovine serum albumin (BSA), and their covalent binding to the protein are reported. Fluorescence and excitation spectra of BA photo-products covalently bound to BSA exhibited considerable changes when compared with the parent hydrocarbon BA. At least two distinct photoproducts with overlapping excitation and fluorescence spectra were formed. The excitation spectrum for the 370 nanometer (nm) fluorescence showed the highest peak at 301 nm, whereas for the 390 nm fluorescence the 326 nm peak was the highest. Using the same purification method, BSA with covalently bound DMBA or MC photoproducts was isolated from UV (365 nm)-irradiated DMBA or MC-BSA complexes. Compared to the BA photoproducts, the DMBA photoproduct had less complicated spectra, exhibiting an excitation peak at 320 nm and fluorescence peaks at 377 and 390 nm. In contrast to DMBA, two photoproducts of MC bound to BSA were formed. One photoproduct had fluorescence peaks at 365 and 380 nm with an excitation peak at 320 nm. While the fluorescence characteristics of a phenanthrene-type product from MC were not available, it was expected to have more blue-shifted excitation and fluorescence maxima. The other photoproduct had fluorescence and excitation peaks almost identical with those of MC, suggesting preservation of the original conjugated double bond system. Results suggested that BA, DMBA, and MC can undergo photo-activation and macromolecular binding as in metabolic activation. (19 Refs)

117. THE COMPLETE AMINO ACID SEQUENCE OF THE MURINE TRANSPLANTATION ANTIGEN H-2DB AS DEDUCED BY MOLECULAR CLONING.

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Immunogenetics; 16(1):1-9 1982

A mouse cDNA library derived from the EL4 cell line (beta-haplotype) was screened with a probe containing a small part of the H-2Kb coding region. One of the clones isolated, pH203, encodes a protein whose deduced amino acid sequence is identical with the known sequence of H-2Db in 141 of 141 positions available for comparison. The clone, therefore, is believed to code for the H-2Db transplantation antigen. The cDNA insert of pH203 contains the coding region for residues 82 through the carboxy-terminus of H-2Db, and includes 476 nucleotides of the 3'-untranslated sequence. Comparison between the H-2Db cDNA clone and a previously isolated H-2Kb cDNA clone shows homologies of 83% and 91% at the amino acid and nucleotide levels, respectively. Analysis of DNA sequences at the 3'-coding and untranslated regions suggests that the mRNAs of H-2Kb and H-2Db are spliced differently at their 3'-coding ends. (Author abstract) (32 Refs)

118. PRIMARY STRUCTURE OF THE H-2DB ALLOANTIGEN. II. ADDITIONAL AMINO ACID SEQUENCE INFORMATION, LOCALIZATION OF A THIRD SITE OF GLYCOSYLATION AND EVIDENCE FOR K AND D REGION SPECIFIC SEQUENCES.

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Immunogenetics; 16(1):11-22 1982

The complete amino acid sequence of the CNBr fragment comprising residues 229-284 of the murine major histocompatibility complex antigen H-2Db has been determined using radiochemical methodology. The sequence was determined by N-terminal sequence analysis of the intact CNBr fragment and by sequence determinations of peptides derived from this fragment by trypsin and staphylococcal V8 protease cleavage. In addition to the amino acid assignments for H-2Db, it was possible to assign the linkage position of the third N-lined glycosyl unit to the asparagine at residue 256. Additional amino acid sequence assignments have also been made for three other CNBr fragments that span residues 99-138, 139-228, and 308-331 of the H-2Db molecule. The total protein sequence information available (222 of 338 residues) agrees in every comparable position with the protein sequence derived from the cDNA clone (pH203) isolated by Reyes and co-workers (1982b), which strongly suggests that this clone encodes H-2Db. Combination of the protein sequences with that deduced from the cDNA clone provides the complete H-2Db protein sequence. Comparison of this sequence with other available protein sequence information for murine Class I molecules has revealed protein sequences that may be unique to either K or D region molecules. (Author abstract) (39 Refs)

119. ACTIVATION OF A B CELL TUMOR WITH LPS (MEETING ABSTRACT).

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Scand J Immunol; 15(1):126 1982

A murine B cell tumor line, BCL, can be activated by the polyclonal B cell activators LPS (lipopolysaccharide) and lipoprotein (LP) from Escherichia coli. As a result of activation, the tumor cells commence IgM secretion, as detected by plaque formation using the protein A plaque assay. The dose response curve using either LPS or LP is broad, even when newly cloned tumor cells are assayed, and quite similar to the dose response curve of normal murine spleen cells. These data suggest that the BCL and perhaps also normal B cells do not bind LPS and LP selectively, but that nonspecific binding leads to triggering via selective sites in the cell membrane. LPS but not LP activation is inhibited by polymixin B. Furthermore, purified lipid A is also a potent activator for BCL cells. This indicates that LP and LPS activation occurs through similar mechanisms as in normal B cells. As with normal B cells, BCL activation is inhibited by rabbit anti-IgM antibodies. Preliminary evidence indicates that this inhibition is mediated via the Fc fragment of the antibodies. (no Refs)

120. MAPPING OF ALPHA-ANTITRYPSIN LOCUS (PI) TO CHROMOSOME 14 IN RAT HEPATOMA X HUMAN FETAL LIVER CELL HYBRIDS (MEETING ABSTRACT).

Pearson SJ, Tetri P, Francke U

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Pediatr Res; 16(4,part2):194A 1982

In order to study the involvement of human chromosomes in the expression of human liver-specific functions, we have produced somatic cell hybrids between a rat hepatocarcinoma cell line and diploid cells from a humam fetal liver. The rat hepatoma line was HPRT (hypoxanthine phosphoribosyltransferase) deficient, and hybrids were selected in medium containing H and ouabain. The presence of human liver-specific proteins was analyzed by immunoelectrophoresis techniques applied to concentrated serum-free hybrid culture supernatants. A subset of hybrids secreted an antigen that was immunologically identical to human alpha 1-antitrypsin (PI). Neither parental line supernatant, no fetal calf serum, nor normal rat serum reacted with this anti-serum. We conclude that interaction of the rat hepatoma genome with that of the human fetal liver cells has activated the human PI locus. In 19 primary HAT (hypoxanthine, amethopterin, thymidine)-selected and 5 azaguanine back-selected hybrids, human PI production segregated concordantly with chromosome 14. All other chromosomes were excluded by discordant clones. Assignment of the PI gene to chromosome 14 is consistent with data of others localizing the GM immunoglobulin heavy chain gene cluster to this chromosome since family studies have established linkage between PI and GM. (no Refs)

121. SV40 A PROTEIN (T ANTIGEN) PHOSPHORYLATION.

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Diss Abstr Int [B]; 42(11):4282-B 1982

A non-specific protease mapping procedure was developed to aid in the analysis of individual phosphorylation sites of the A protein. The A protein is phosphorylated at five or more sites. One phosphothreonine and four phosphoserine sites were distinguished. Several phosphorylation sites were mapped using T antigens encoded by deletion mutants. This method can be used to examine differentially phosphorylated A proteins in attempts to correlate structure with function. Examination of tsA58 A protein synthesized at the restrictive temperature demonstrated that phosphorylation occurred at the same sites as in wild type A protein, but a quantitative difference was seen in the phosphorylation of one phosphopeptide. Dephosphorylation did not affect the ability of the A protein to bind specifically to SV40 origin DNA. No differences were seen in the location, alignment, or affinity of the binding. It is, therefore, unlikely that phosphorylation at the majority of the phosphoserine sites is directly involved in the regulation of site-specific recognition and binding of the A protein to SV40 DNA. (no Refs)

122. METABOLIC ALTERATIONS IN LIVER, SKELETAL MUSCLE, AND FAT TISSUE IN RESPONSE TO DIFFERENT TUMOR BURDENS IN GROWING SARCOMA-BEARING RATS.

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J Surg Res; 33(1):23-31 1982

Metabolic alterations in growing sarcoma-bearing rats were studied and are presented in relationship to a small and moderate tumor burden (5 and 10% dry tumor weight of dry carcass weight). Sarcoma-bearing rats with a small tumor burden had decreased plasma concentration of glucose, significantly changed hepatic protein metabolism, increased lipolysis in fat tissue, but not significantly changed protein metabolism in skeletal muscles. Tumor-bearing rats with a moderate tumor burden had altered hepatic protein metabolism and plasma glucose in a manner similar to rats with a small tumor and similar to that previously reported for nongrowing sarcoma-bearing mice with a large tumor. This was also similar to findings in cancer patients. In contrast to sarcoma-bearing mice and man, these rats with a 'moderate tumor' still had unchanged nitrogen balance in skeletal muscle muscle. We conclude that metabolic alterations in the liver precede catabolic reactions in peripheral tissues and that initiating factors for increased lipolysis are earlier than those factors initiating wasting of skeletal muscles in growing sarcoma-bearing rats. (Author abstract) (38 Refs)

123. CONTROL OF ACID PHOSPHATASE ACTIVITY IN HUMAN PROSTATIC CARCINOMA CELL CULTURES BY SERUM.

Kirk D, Narayan KS, Vener G, Kaighn ME, Van Etten RL, Shaw LM

Pasadena Foundation for Medical Res., Pasadena, CA, 91101

Ann NY Acad Sci; 390:62-72 1982

Effect of serum on the expression of acid phosphatase (AP) activity in prostatic carcinoma cell lines PC-3 and DU-145 was investigated. The two cell lines were maintained in nutrient medium PFMR-4 supplemented with 7% fetal calf serum, and their sublines (PC-3SF12, DU 145SF1) were cultured in PFMR-4 without serum. AP activity was significantly higher in cells maintained without serum. In the presence of serum, the activity of PC-3 cells was seven-fold lower when based on cell number or protein and four-fold lower on a DNA basis. The effect of tartrate (10 milliM) on AP activity of PC-3 and DU 145 with and without serum was also tested. The results showed that only approx 40% of the activity detected in serum-maintained cultures of both lines was sensitive to tartrate inhibition. When cells that had been growing in serum-free PFMR-4 were switched to serum-supplemented medium, serum decreased AP activity by more than 60% based both on protein and DNA content. The effect of serum on the AP activity in PC-3SF12 was also studied by a histochemical method. Localized enzyme activity was markedly decreased by addition of serum. Morphological effects of serum were examined by scanning electron microscopy. The morphology and surface topography of PC-3SF12 cells supplemented with serum for 5 days were intermediate between those of PC-3 and PC-3SF12 suggesting that the alterations are reversible. The mechanism by which serum inhibits AP activity in prostatic carcinoma cells is not known. (21 Refs)

124. MECHANISMS THAT UNCOUPLE GROWTH AND DIFFERENTIATION IN MYELOID LEUKEMIA CELLS: RESTORATION OF REQUIREMENT FOR NORMAL GROWTH-INDUCING PROTEIN WITHOUT RESTORING INDUCTION OF DIFFERENTIATION-INDUCING PROTEIN.

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Proc Natl Acad Sci USA; 79(14,part1):4347-4351 1982

There are different macrophage- and granulocyte-inducing (MGI) proteins. Normal myeloid precursors are induced to multiply by one form (MGI-1) and to differentiate by another form (MGI-2). There are clones of myeloid leukemia cells that no longer require MGI-1 for growth but can still be induced to differentiate by MGI-2. After induction of differentiation in these leukemia cells by adding MGI-2 or inducing endogenous production of MGI-2 by lipopolysaccharide, the differentiating leukemia cells, like normal cells, again required MGI-1 for growth. This growth requirement for MGI-1 could not be substituted for by adding other protein growth factors such as epidermal, fibroblast, or nerve growth factor or insulin. Induction of differentiation in these leukemia cells by dexamethasone, arabinonucleoside (cytosine arabinoside), or methotrexate instead of by MGI-2, did not restore the requirement of MGI-1 for growth. Mutant myeloid leukemia cells that could not be induced to differentiate by MGI-2 also did not show this restoration of the requirement of MGI-1 for growth. MGI-1 in normal cells induced cell growth and also induced MGI-2, so that the cells could then differentiate by the endogenously produced MGI-2. However, MGI-1 did not induce production of MGI-2 in the leukemia cells, even though they again required MGI-1 for growth, so that there was no induction of differentiation after adding MGI-1. This lack of induction of differentiation-inducing protein by growth-inducing protein has thus identified an effective mechanism for uncoupling of growth and differentiation in malignant cells. (Author abstract) (43 Refs)

125. SEROLOGICAL ANALYSIS OF CELL SURFACE ANTIGENS OF NULL CELL ACUTE LYMPHOCYTIC LEUKEMIA BY MOUSE MONOCLONAL ANTIBODIES.

Ueda R, Tanimoto M, Takahashi T, Ogata SI, Nishida K, Namikawa R, Nishizuka Y, Ota K
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Proc Natl Acad Sci USA; 79(14,part1):4386-4390 1982

Nine antigen systems were defined. Two were related to HLA-A,B,C and to Ia-like antigens; the others could be grouped into three categories. (i) NL-22, NL-1: NL-22 antibody reacted with leukemia cells from 12 of 16 cases of null cell acute lymphocytic leukemia (null-ALL) but not with any other type of leukemia tested or with lymphoid cells of various origins. Among cultured cell lines tested, one (NALM-6) of three null-ALL cell lines was positive; the others were negative. Absorption analysis confirmed the restriction of NL-22 antigen to null-ALL. NL-1 antibody was reactive with leukemia cells from 10 of 16 cases of null-ALL and 3 of 6 cases of chronic myelocytic leukemia in blastic crisis (CML-BC). The antigen was present also on a minor population of normal lymphoid cells. The distribution and mol wt (100,000; glycoprotein) of the NL-1 antigen resembled that of the previously described common ALL antigen (cALL). (ii) NL-30, NL-4: Both antibodies exhibited almost identical patterns of reactivities against cultured cell lines tested. They reacted with leukemia cells from some cases of null-ALL, adult T-cell leukemia, and CML-BC, although they showed discordance in their reactivities against a panel of leukemia cells. (iii) NL-9, NL-8, NL-25: These three antibodies detect serologically distinguishable determinants on a broad range of leukemias and normal lymphoid and hematopoietic cell types. The antibodies analyzed in this study provide evidence for the heterogeneity of null-ALL by demonstrating a variety of antigen phenotypes on leukemia cells. One of the antigens (NL-22) appears to be restricted to null-ALL. (Author abstract) (27 Refs)

126. THE ROLE OF LIVER IN THE CATABOLISM OF HUMAN ALPHA- AND BETA-INTERFERON.

Bocci V, Pacini A, Bandinelli L, Pessina GP, Muscettola M, Paulesu L

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J Gen Virol; 60(part2):397-400 1982

The susceptibility of human leukocyte alpha interferon, fibroblast beta interferon and recombinant alpha-2-interferon to clearance by the isolated and perfused rabbit liver has been evaluated. Human leukocyte and recombinant alpha-2-interferons were stable and their initial levels were maintained in the perfusate even if they had been treated with neuraminidase, thus suggesting that alpha-interferons have no exposed sugars recognizable by hepatic binding proteins. On the other hand, native, and particularly desialylated human beta-interferon, underwent marked hepatic uptake confirming the importance of the liver as a catabolic site for glycosylated interferons. (Author abstract) (21 Refs)

127. EXPRESSION OF PHENYLALANINE HYDROXYLASE ACTIVITY IN HUMAN HEPATOMA CELLS (MEETING ABSTRACT).

Hawkins M, Jackson WB, Brown TE

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Fed Proc; 41(3):1401 1982

Phenylalanine hydroxylase [Phe-OH, EC 1.14.16.1] is a liver enzyme which catalyzes the irreversible hydroxylation of phenylalanine to tyrosine in mammalian cells. Enyzme specific activity was determined by assaying the rate of conversion of 14C-phenylalanine to 14C-tyrosine in cell extracts as distinguished by TLC (thin layer chromatography) on cellulose plates. Hepatoma lines PLC/PRF/5, Hep 3B and Hep G2 had substrate conversion rate of 33-44% with activities of 1.94, 2.03, and 1.90 nmoles tyr/min/mg protein, respectively. Hepatoma line SK-HEP-1 had no measurable enzyme activity and also does not have the typical hepatoma cell morphology. Inducible enzyme activity is increased two-fold in the presence of 10 uM hydrocortisone, and is inhibited 78-84% by 5 mM p-chlorophenylalanine. 5-Iodotyrosine (Tyr) (10 uM, inhibits tyrosine hydroxylase) and 6-fluorotryptophan (0.1 mM, inhibits tryptophan (TrP) hydroxylase) did not inhibit enzyme activity at the concentrations shown. Cells grown in F14 tyr-free medium with dFCS (fetal calf serum) show a slight decrease in activity. These results show that cells in culture do express Phe-OH activity, and that the conversion of phe to tyr is mediated by Phe-OH and not by Tyr-OH or Trp-OH. (no Refs)

128. DIRECT INHIBITION OF THE CATALYTIC PROTEIN OF ADENYLATE CYCLASE AT THE ADENOSINE P SITE (MEETING ABSTRACT).

Florio VA, Ross EM
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Fed Proc; 41(3):1408 1982

Both adenosine and the P site agent 2',5'-dideoxyadenosine (DDA) reversibly inhibit the activity of the catalytic protein (C) of adenylate cyclase either in a resolved C fraction from hepatic plasma membranes or in plasma membranes of cyc- S49 lymphoma cells. Both preparations are free of the GTP(guanosine triphosphate)-binding regulatory protein, G/F. Inhibition by DDA is insensitive to 0.4 mM 1-methyl-3-isobutylxanthine or adenosine deaminase (2.5 IU/ml). The low basal activity of C in the presence of Mg++ is slightly inhibited. Mn++-stimulated activity (6-10 x basal) is inhibited up to 40%. Further reversible stimulation by Mn++ plus forskolin (10-30 x Mn++) or quasi-irreversible stimulation by GTP-gamma-S-preactivated G/F (5-20 x Mn++) are completely blocked by Ado or DDA. There is no effect of submaximal amounts of DDA on the concentrations of Mn++, forskolin, or GTP-gamma-S-preactivated G/F needed for activation of C, nor is there an effect of any activator on the concentration dependence of inhibition by DDA. DDA does not alter the rate of activation of G/F by GTP-gamma-S nor does it promote dissociation of the stable G/F-C complex formed in the presence of GTP-gamma-S. DDA is not competitive with substrate, as shown by others. These data suggest that DDA acts directly on C, but that it does not bind or regulate C when activating ligands are absent. (no Refs)

129. INHIBITION OF MOUSE MASTOCYTOMA PROTEIN KINASES BY AMILORIDE.

Ralph RK, Smart J, Wojcik SJ, McQuillan J
Dept. Cell Biology, Univ. Auckland, Auckland, New Zealand
Biochem Biophys Res Commun; 104(3):1054-1059 1982

In recent studies on the control of growth of PY815 mouse mastocytoma cells by cyclic (c)AMP, it was found that amiloride (AM; a potent inhibitor of Na+ transport) inhibited both Type I and II cAMP-dependent protein kinases prepared from PY815 cells. These studies suggest that AM may affect growth by inhibiting protein phosphorylation and hence cation transport. Increasing concentrations of AM or ouabain progressively inhibited growth at concentrations in excess of 1 x 10(-5) M, indicating a requirement for Na+ transport for continuous growth. In an in vitro assay, increasing concentrations of AM inhibited histone phosphorylation by type I and II cAMP-dependent protein kinases prepared from mastocytoma cells. Histone phosphorylation was inhibited over approx the same AM concentration range that affected growth. Ouabain was without effect. AM (1 milliM) did not affect ATP-45Ca++ uptake by membranes from mastocytoma cells and incubated with azide and oxalate to eliminate effects of residual mitochondria. AM did not prevent the stimulation of 45Ca++ uptake that resulted when the membranes were first preincubated with type I or II protein kinases. Thus, pre-phosphorylating isolated membranes appeared to activate a new route for 45Ca++ transport and inhibiting the kinase with AM prevented formation of this new Ca++ channel. Results suggest that AM-sensitive ion transport channels in cells may be controlled by protein phosphorylation which alters the availability of intracellular cations to affect growth. (22 Refs)

130. INTERACTION OF N-ETHYL-N'-NITRO-N-NITROSOGUANIDINE WITH NUCLEIC ACIDS AND PROTEINS IN COMPARISON WITH N-METHYL-N'-NITRO-N-NITROSOGUANIDINE.

Yoda K, Sakiyama S, Fujimura S
Div. Biochemistry, Chiba Cancer Center Res. Inst., 666-2 Nitona-Cho, Chiba 280, Japan
Chem Biol Interact; 41(1):49-59 1982

Reactivity of N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) was studied in comparison with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The radioactivity of [guanidino-14C]ENNG was incorporated only into the protein fraction and that of [ethyl-14C]ENNG was incorporated into DNA, RNA and protein fractions in ascites hepatoma AH7974 cells, as were those of [guanidino-14C]- and [methyl-14C]MNNG, respectively. The amounts of the binding of ENNG were less than those of MNNG, especially in the incorporation of the ethyl moiety of ENNG into nucleic acid fractions. In a noncellular system, the radioactivity of [guanidino-14C]ENNG was incorporated into proteins, preferentially into basic proteins such as cytochrome c, but was not incorporated into nucleic acids. This behavior is similar to that of [guanidino-14C]MNNG, while the amount of binding of the former was about half of that of the latter. The radioactivity of [ethyl-14C]ENNG was also incorporated into basic proteins to almost the same extent as that of [methyl-14C]MNNG. However, the binding of the ethyl moiety of ENNG to nucleic acids was much lower than that of the methyl moiety of MNNG. Horse heart cytochrome c, bovine pancreatic RNase A and regenerating rat liver chromatin had altered their biological activities to various degrees after modification by ENNG or MNNG. (Author abstract) (25 Refs)

131. CORRELATION BETWEEN IMMUNOGLOBULIN LIGHT CHAIN EXPRESSION AND VARIANT TRANSLOCATION IN BURKITT'S LYMPHOMA.

Lenoir GM, Preud'homme JL, Berheim A, Berger R
International Agency for Res. on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 2, France
Nature; 298(5873):474-476 1982

The relationship between the expression of immunoglobulin chains in Burkitt's lymphoma (BL) cells in 19 continuous lines and 10 fresh specimens was studied in relation to observed chromosomal anomalies. Of the 19 cell lines used, 9 contained a t(8;14) translocation, 5 had a t(8;22), and 5 had a t(2;8). Nine of the fresh specimens had t(8;22) and one had t(8;22). Immunoglobulin expression varied from a pre-B to mature B cell phenotype. Translocation t(2;8) may be associated with a relatively immature phenotype, or at least with poor light chain expression, as pre-B or transitional pre-B/B phenotypes were found in 3/5 cases with the t(2;8) translocation, but in only 1/8 with t(8;22) and 1/19 with t(8;14). Marked differences in the type of light chain were observed, depending upon the type of translocation. When a light chain is expressed in BL cells, its type is strictly related, in cases of variant translocation, to the chromosomes involved: kappa in t(2;8) translocation and gamma in t(8;22) translocation. Light chain types were equally distributed among cases with t(8;14). The correlation between immunoglobulin expression and chromosomal rearrangements found in the present study strongly suggests that following chromosomal translocation an immunoglobulin gene and a putative oncogene become adjacent, and are simultaneously activated under the influence of the same transcription element. (19 Refs)

AUTHOR INDEX WITH CITATION NUMBERS

Abramson M 76
Adkins B 41
Adolf GR 113
Agius C 46
Albini A 66
Alcock SR 99
Allan BJ 15
Alvares AP 65
Ashendel CL 49
Ashitaka Y 40
Bachur NR 82
Back N 36
Bagshawe KD 101
Baker MA 78
Balwierczak J 36
Bandinelli L 126
Barron M 73
Barsky SH 83
Basu M 81
Basu S 81
Baum LL 48
Baylin SB 55
Bedi GS 36
Beiderman B 67
Berger R 131
Berheim A 131
Bernal SD 55
Bettelheim P 64
Bickers DR 43
Blitzer A 76
Bocci V 126
Bohmer FD 84
Boughton BJ 62
Boukamp P 10
Bourne HR 67
Boutwell RK 49
Breitkreutz D 10
Brooman P 42
Brothers VM 67
Brown DM 80
Brown TE 127
Browne P 101
Bunzli I 18
Bu ghouts JT 90
Burrow-Meckbach 18
Camitta BM 114
Cantor J 106
Carmichael MJ 88
Carnegie GE 99
Carraway KL [illegible]
Cartei G
Castellino FJ [illegible]
Catterall WA 104
Cavanaugh PG 75
Chan SY 25
Chawla RK 27
Cheng L 19
Chiasson MA 26
Cho-Chung YS 89
Choe BK 73
Chou JY 105
Chung AE 92
Claque MB 88
Codington JF 69
Coffey T 51
Cohen AB 47
Coligan JE 118
Cossman J 52
Costa MR 104
Craddock VM 1
Curtoneo KR 107
De Ceccatty MP 60
DeJong JG 56
Dekker AW 56
DiAugustine RP 17
DiPetrillo TA 107
Dixit R 43
Dobrjansky A 26
Dong MK 73
Drings P 18
Durkin ME 92
Duvic CR 34
Edstrom S 122
Edwards EH 79
Ekman L 122
Ekstrom G 111
Enrietto PJ 97
Erikson RL 97
Evens C 75
Fahlbusch B 23
Falk JA 78
Faucon M 60
Felix JS 58
Felsted RL 82
Fiorentino M 28
Fischer P 113
Fischer S 5
Fischkoff S 82
Fiszman MY 5
Florio VA 128
Francke U 120
Franke WW 9
Freake HC 54
Friis R 14
Frixen U 14
Fujimori E 116
Fujimura S 130
Fujiyama Y 21
Furcht LT 61
Fusenig NE 10
Gacon G 5
Gallagher RE 82
Gallaher WR 34
Gash S 1
Gazdar AF 55
Gersten DM 39
Gewurz H 48
Gibert MA 60
Gidari AS 46
Gilbert JA 2
Gisselbrecht S 5
Glaumann H 111
Gleason S 73
Glover CJ 82
Goeghegan TE 93
Gordon J 38
Grant S 78
Green S 26
Griffin MJ 44
Griffin MM 42
Gropp C 16
Guerra C 28
Gupta SK 82
Gustafsson JA 112
Haas OA 113
Hachiya T 21
Haeberli A 29
Hales CN 45
Hall IH 100
Halper JP 103
Hamamoto T 40
Hamazu M 21
Hanash SM 57
Hannam-Harris A 38
Hanson-Painton 44
Hare JD 13
Havemann K 16
Hawkins M 127
Hearing VJ 39
Henderson AR 1
Heyn RM 57
Hill GL 115
Hoffee PA 108
Honn KV 75
Hosoda S 21
Huang CC 76
Huang FL 89
Hulley TP 42
Humphries DE 33
Hunt SW 108
Hunter T 41
Hunter T 98
Hutton JC 45
Imura H 95
Ingelman-Sundbe 111
Ishida M 21
Jackson WB 127
Jacob HS 61
Jahnke GD 17
James K 48
Jannotta F 83
Johnston ID 88
Kahan BD 70
Kaighn ME 123
Kaizu T 71
Kaizu T 72
Kajita Y 21
Kao R 24
Kapteijn R 56
Karlberg I 122
Kartha S 58
Kasuga M 20
Kay NE 61
Keir MJ 88
Kibert LG 3
Kien CL 114
King VL 13
Kinoshita F 95

Kirk D 123
Klein DJ 80
Klein G 69
Klein KJ 32
Kline LE 47
Knapp W 64
Knoop SJ 93
Knowles AF 86
Koerner TJ 53
Kojima O 20
Koller R 12
Konrad K 30
Kotch JP 107
Krieg T 66
Kurimoto T 20
Kyle JW 81
Labant MC 108
Laine RA 71
Laine RA 72
Lambert MW 4
Lambert WC 4
Lazarus LH 17
LeGrue SJ 70
Lee DE 4
Lee KH 100
Lenoir GM 131
Levinson C 79
Lewin K 19
Lewis NJ 85
Lillehoj HS 73
Lindmark L 122
Linebaugh BE 94
Liotta LA 83
Liou YF 100
Liska L 64
Lotem J 124
Lubec G 31
Lundholm K 122
Luster W 16
MacIntyre I 54
MacNeil S 68
Macek CM 70
Mackiewicz A 22
Majdic O 64
Majima S 20
Maloy WL 118
Mandl I 106
Manke HG 18
Marchalonis JJ 39
Matsuda T 63
Matthews RH 85
McCarthy J 61
McMaster GK 59
McMichael TL 85
McQuillan J 129
Meigel W 66
Melcher UK 91
Melewicz FM 47
Mensing H 66
Meyer RR 53
Michael AF 80
Miller SC 69
Milo GE 85
Minna JD 55
Mirkin BL 6
Mohanakumar T 51
Mohanakumar T 78
Moll R 9
Mombelli G 29
Morimoto N 40
Moschl P 31
Mukhtar H 43
Muller HK 7
Muller P 66
Murray MD 34
Muscettola M 126
Myazaki T 21
Nakai Y 95
Nakao K 95
Namikawa R 125
Narayan KS 123
Neckers LM 52
Neel JV 57
Nilsen OG 112
Nishida K 125
Nishimura R 40
Nishioka B 20
Nishizuka Y 125
Noel P 60
O'Dea RF 6
O'Donnell-Torme 77
O'Leary MC 6
Ochi Y 21
Oegema TR 80
Ogata SI 125
Oki S 95
Okorodudu AO 4
Olstein AD 74
Osborne R 102
Osman M 106
Ota K 125
Ozawa M 40
Pacini A 126
Papkoff J 98
Paulesu L 126
Pearson GR 2
Pearson SJ 120
Pengo V 28
Penn EJ 45
Pennington TH 99
Pernis B 103
Perri RT 61
Pessina GP 126
Piau JP 5
Pikler GM 2
Plas AM 90
Pollard M 25
Pontz B 66
Preud'homme JL 131
Purchio AF 97
Ralph RK 129
Rausch DJ 27
Reber HA 15
Reyes AA 117
Rillema JA 94
Roberts C 15
Rockley NL 91
Roffman S 106
Roheim PS 34
Roncari DA 78
Rose NR 73
Rosenblum BB 57
Ross EM 128
Roth P 103
Roux A 29
Ruscetti S 12
Rush J 72
Rush JS 71
Russell EC 51
Sachs L 124
Sakiyama S 130
Salden MH 90
Samtleben R 84
Sandy L 57
Schenk P 30
Schersten T 122
Schilling JJ 15
Schold M 117
Schon R 84
Schulz U 9
Schumann I 23
Sculley TB 2
Sefton BM 41
Severinson E 119
Shah H 8
Shaper JH 55
Shaw LM 123
Shaw SA 121
Shenton B 88
Shoyab M 50
Siegal GP 83
Simpson A 62
Sixma JJ 56
Sledge WE 34
Sloane BF 75
Smart J 129
Smith GM 34
Smith JL 38
Spelsberg TC 2
Spiegelberg HL 47
Springstead KN 57
Steinberg F 67
Sterling KM 107
Stern R 24
Steven FS 42
Stevens GS 57
Straub PW 29
Streumer-Svobod 87
Strickland DK 25
Surrey S 110
Sutton HE 96
Swetly P 113
Szalay S 31
Takahashi T 125
Tanaka I 95
Tang JN 44
Tanimoto M 125
Tashjian AH 102
Taub RN 78
Taylor I 19
Tetri P 120
Thompson JJ 34
Tilgen W 10
Todaro GJ 50
Toftgard R 112
Tojo S 40
Toomes H 18
Tu YY 109
Tubergen DG 57
Turco SJ 71
Turco SJ 72
Turino GM 106
Tyrey L 11

Ueda R 125
Uehara Y 20
Ueng TH 65
Ulich T 19
Van Dongen AA 87
Van Etten RL 123
Van Wijk R 87
Vener G 123
Vessella RL 61
Vetter ML 48
Von Bahr C 111
Von Bassewitz D 9
Von der Helm K 14
Wallace RB 117
Watanabe S 20
Weickmann JL 35
Weinhouse S 8
Weinstein IB 103
Wessels HM 90
White TT 15
Wiegant FA 87
Wille W 14
Willecke K 14
Wojcik SJ 129
Wolff L 12
Wong M 24
Word CJ 37
Wray LK 96
Wright DH 38
Wu CH 76
Yamane T 20
Yang CS 109
Yang K 19
Yasutomi M 63
Yoda K 130
Yoshida C 8
Zardi L 59
Zschiesche W 23

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